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Intraspecific differences in the response of perennial ryegrass (*Lolium perenne* L.) to drought

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Samuel Symes Wilson

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Abstract of a dissertation submitted in partial fulfilment of the requirements for the Degree of Bachelor of Agricultural Science with Honours

Intraspecific differences in the response of perennial ryegrass (*Lolium perenne* L.) to drought

By

Samuel Symes Wilson

Perennial ryegrass (*Lolium perenne* L.) has traditionally been the grass species of choice for most New Zealand regions and is currently an important component of our highest-producing pastures. However perennial ryegrass is drought-sensitive and recent climate change projections for increased drought frequency and intensity in New Zealand have raised industry concern about the limitations of perennial ryegrass, particularly for northern and eastern regions. One possible solution is the introgression of wild-type germplasm that possess morphological and physiological attributes which improve drought tolerance, into high-producing commercial cultivars of perennial ryegrass. However there has been little research on perennial ryegrass in New Zealand that includes morphological and physiological drought response mechanisms. The objective of the current study was to investigate the responses to drought, of two bred perennial ryegrass cultivars; Grasslands Samson and Cropmark Seeds Kai; and two ecotypes, one of Norwegian origin and the other from Tunisia. This research was carried out in a glasshouse at Lincoln University, Canterbury during the winter of 2016.

Under favourable conditions, the bred populations out-performed the ecotypes, producing 43% greater shoot biomass over the whole experiment than the ecotypes. The drought treatment reduced the shoot biomass of all populations, resulting in comparable shoot biomass production under drought among the four populations. However the Norwegian ecotype, in relation to its well-watered plants, was reduced by 58% by drought, showing a smaller reduction than Kai

(-70%). Leaf DM (dry matter) production and LER (leaf extension rate) were both reduced by drought treatment in all populations. The mean LER and leaf DM production of the Tunisian population under the second drought by -58% and -23% respectively was a smaller reduction than that experienced by Kai (-70% and -64% respectively). The mean LER of the Norwegian population was reduced to the largest degree in the first drought cycle (-85%). The root to shoot DM ratio was increased in Kai, Samson and the Tunisian population under drought and to the greatest degree in the Tunisian population (+88%). However the root to shoot DM ratio of the Norwegian population remained unaffected by the drought treatment. The RWC (relative water content) across all populations was halved by the drought treatment, from a mean RWC of 92% in the well-watered plants to 47% under drought. However, the Norwegian population was able to maintain a greater RWC (60%) than that of Kai in the drought treatment (35%). The solute potential was reduced by drought in Kai (-145%), Samson (-139%) and the Tunisian population (-92%), but was unchanged in the population from Norway, indicating that it did not osmotically adjust. There were no differences in the chlorophyll concentration or leaf temperature between the four populations under drought. The Tunisian population was the only endophyte-infected population of the four.

Taken together, this study identified intraspecific differences in key morphological and physiological drought responses of perennial ryegrass. The ecotypes showed signs of early drought tolerance, in contrast to the bred populations. Furthermore, there were differences in the drought tolerance strategies between the ecotypes, where the Norwegian population reduced leaf growth and preserved resources, and the Tunisian population increased its root to shoot DM ratio and was able to maintain LER to a greater extent than the other populations under drought as a result. The presence of endophyte in the Tunisian population likely contributed to its drought tolerance strategy. These findings provide functional information that can be used in future breeding initiatives towards improving drought tolerance in perennial ryegrass.

Keywords: perennial ryegrass, drought, drought tolerance, response mechanisms

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1 Introduction

Perennial ryegrass (*Lolium perenne* L.) is the major grass species in New Zealand's most fertile and high-producing pastures and is commonly the greatest dietary component of our most intensively grazed livestock (Easton et al. 2001). It is the most commonly sown pasture grass in New Zealand (Kemp et al. 2002) and around the world (Langer 1990; Turner et al. 2006). However the production and persistence of perennial ryegrass is often reduced when exposed to dry conditions and pests (Parry et al. 1992; Turner et al. 2006). The competitiveness of perennial ryegrass in a mixed sward may also become reduced by water deficit (Thomas 1984).

New Zealand has a temperature climate ideally suited to pastoral agriculture, however, while annual rainfall exceeds 1000mm annually in most regions, variability between seasons is large, with 20% of years being considered as wet and 15% of years being considered as dry (Barker et al. 1985). Additionally, the increased incidence of drought evens since 1968, in combination with the geographical variability of rainfall, has led to drier conditions in the east and wetter conditions in the west (Salinger 1979). Based on recent climate change predictions, the regional variability in annual rainfall is likely to increase and exacerbate moisture deficits in northern and eastern regions of the country (Hollis 2014). Additionally rising temperatures are likely to increase rates of evapotranspiration considerably, adding to the increased likelihood and frequency of soil moisture deficits (Salinger 2003).The combination of limited water supply and climate change could reduce the dominance of perennial ryegrass-based pastures in New Zealand (Moloney 1991).

The development of perennial ryegrass cultivars with improved water deficit tolerance traits could represent a solution for affected regions (Matthew et al. 2012). However, despite the relatively extensive knowledge on general drought tolerance mechanisms of plants, and the impact of drought on the morphology of perennial ryegrass, there is little knowledge about the physiological responses of perennial ryegrass to drought in New Zealand. The knowledge gap therefore restricts the rate of improvement in perennial ryegrass under drought because (i) there is a lack of clarity in which breeding traits will have the most beneficial effects on yield under drought and (ii) it is unclear which germplasm sources are going to provide the desired traits (Matthew et al. 2012).

Commercial perennial ryegrass cultivars are traditionally bred for pasture production on high fertility soils and in moist conditions (Charlton & Stewart 1999). However the performance of perennial ryegrass over the summer months and in drier regions is often poor and the ryegrass

proportion of these pastures commonly declines following prolonged water deficit (Harris et al. 1985). The introgression of drought tolerant genotypes into high performing commercial cultivars may provide one solution. Several studies have found that wild-type perennial ryegrass populations possess such genotypes, however yield is typically low under favourable conditions and is often substituted for the protective response mechanisms under drought (Hussain 2013). The persistence, under drought, of these ecotypes is likely to be improved, as they focus more energy into survival mechanisms to overcome the drought (Lelièvre & Volaire 2009).

In the current study, we imposed water deficit stress on two commercial cultivars; Grasslands 'Samson' and Cropmark Seeds 'Kai' as well as two ecotypes; one from Norway and one from Tunisia. We anticipated that the ecotypes would be more drought-tolerant than the bred populations. Through the monitoring of key physiological traits with links to improved drought tolerance it was expected that functional differences between the bred populations and ecotypes could be identified.

2 Literature review

New Zealand's unique and extreme topography creates a wide variability in climate between and within the two main islands. As a result, the use of perennial ryegrass is limited to areas with sufficient rainfall and lower average annual temperatures (Hussain 2013). However with impending rises in temperature and changes in the seasonal and regional variability of rainfall as a result of climate change, some areas that have relied on perennial ryegrass-based pastures will likely exhibit a loss in production in the future. Currently, there is concern about the persistence of perennial ryegrass, particularly for warmer and drier regions (Glassey 2011), resulting in a recent focus on the best possible methods for increasing drought tolerance in perennial ryegrass (Matthew et al. 2012). Despite this there has been little investigation into drought tolerance in pasture grasses at the mechanism level.

Important components of drought tolerance for perennial ryegrass are the ability of the species to (i) produce reasonable dry matter yields under moisture deficit and (ii) persist for long periods of time under drought conditions (defined as the stability, or persistence of dry matter yield, after (Parsons et al. 2011)). These factors are a function of environmental, physiological and biochemical parameters and may include key requirements such as water, nitrogen and adequate temperature, which affect plant processes including leaf extension rate, leaf chlorophyll concentration and other cellular-level processes. This section reviews the literature on the potential responses of perennial ryegrass to drought with reference to the mechanisms involved in their growth and strategies for drought tolerance.

2.1 Perennial ryegrass

Perennial ryegrass (*Lolium perenne* L.) is a compact grass that has dark-green, hairless leaves with a shiny underside that emerge from its numerous flattened tillers (Langer 1990). Leaves and adventitious roots grow from a compact stem as there is very little internode elongation in the vegetative plant, hence the stem remains below grazing height (Hunt & Field 1976). The fully developed plant produces a bulk of adventitious root of similar thickness and branching to seminal roots, which are comparatively shallow in comparison with other pasture species, which makes the plant vulnerable to water deficit in dry regions (Mayfield & Neilson 1996).

Perennial ryegrass is the most widely used grass for New Zealand pastoral farming as it is easy to establish and manage, grows well in a wide range of soil fertilities and is compatible with White clover (*Trifolium repens* L.) (Charlton & Stewart 1999). With a heavy grazing and pugging tolerance and a capability of producing high annual yields for an extended period of time, ranging from five to 20 years (depending on environment and management), perennial ryegrass provides

the benchmark for comparison with other pasture species in New Zealand (Kerr 1987; Kemp et al. 2002; White & Hodgson 2011). Annual dry matter yields in New Zealand commonly range between 10,000 to 25,000 kilograms of dry matter per hectare in soils of high fertility and animal production is typically high with a digestibility of 75-85% (White & Hodgson 2011). However in regions where rainfall is highly seasonal and summers are warm and dry, growth and persistence is particularly poor (Turner et al. 2006). The optimum temperature for perennial ryegrass is 18°C, with growth occurring from as low as 5°C (White & Hodgson 2011).

For use in this experiment, four populations of perennial ryegrass were selected. This included Grasslands 'Samson', a commonly used New Zealand diploid and; Cropmark Seeds 'Kai', a recently released New Zealand bred tetraploid; Two further populations were sourced from the Margot Forde Germplasm Centre: an ecotype, A 14499, collected in Tunisia and an alternative ecotype, A 17183, collected from Norway.

2.1.1 Grasslands Samson

Samson is a diploid perennial ryegrass bred by AgResearch Grasslands in the 1990's. The breeding programme combined germplasm of the Mangere ecotype (included Grasslands Nui and Ellett) and persistent plants were collected predominantly from direr eastern regions, such as Northland, Hawkes Bay, Waikato and Canterbury. Plants from Taranaki, Manawatu and Southland were also included (Stewart 2006).

Plate 2-1 Grasslands Samson

Samson is a general purpose, medium leafed, medium tillered, semi-erect cultivar with excellent resistance to crown rust. It has a rapid and vigorous establishment and produces high dry matter yields, particularly throughout the summer months (Table 2-1), with reduced stem production during spring months. Annual dry matter production is commonly around 13,000 kg/ha (Easton et al. 2001).

Table 2-1. Common perennial ryegrass cultivars; Samson, Impact and Nui mean seasonal and annual adjusted yields (kg/ha) over all 17 trials conducted by the NZPBRA (New Zealand Plant Breeding and Research Association) throughout New Zealand after 1991. Adapted from (Easton et al. 2001).

	Yield (kg/ha)				
Cultivar	Autumn	Winter	Spring	Summer	Annual
Samson	2800	1600	4625	3850	13000
Impact	2900	1725	4375	4050	13100
*Nui	2625	1550	4500	3575	12350

* Cultivar available before 1991

The NZPBRA trials also illustrate the progress that perennial ryegrass breeders have made in terms of seasonal yield. Perennial ryegrass cultivars released to the market after 1991 yielded 6% more herbage annually and 9% more herbage in summer in comparison with cultivars available before 1991 (Table 2-1) (Easton et al. 2001). The increase in summer herbage yield is indicative of cultivars that may be more tolerant of lower soil moisture. Samson had one of the highest summer herbage yields and yielded relatively better in the North Island, which is possibly a reflection of the origin of the accessions used in its breeding. Samson also had a high spring production, which is critical for meeting increased animal demand during this time.

The incorporation of Samson into sheep, beef and dairy farms across New Zealand has been extremely successful, due to its ability to provide strong and reliable pasture production. As a consequence, Samson has been included in several experiments with a range of objectives (Bryant et al. 2009; Chynoweth et al. 2012; Matthew et al. 2012) . Samson will serve as the New Zealand benchmark cultivar in this investigation.

2.1.2 Cropmark Seed's Kai

Kai is a tetraploid perennial ryegrass recently bred and marketed by Cropmark Seeds. Kai is a densely tillered and late heading perennial ryegrass with fast establishment and rapid regrowth, strong winter activity and high overall seasonal and annual yields. It has a medium-erect growth habit and is well suited to grazing by all livestock types, high pasture quality and good disease resistance.

Plate 2-2 Cropmark Seeds Kai

In the national forage variety trial, annual yields were 14,000 kg/ha, but reached as high as 16,000kg/ha in some years. Kai is a high quality feed and so is better suited to paddocks with good fertility and in areas with reliable rainfall or irrigation. Additionally, because Kai is late heading, it has better late season quality than early maturing perennial ryegrass cultivars. Kai is usually available in low endophyte or nil endophyte. Kai was included in this work because of its nature as recently bred, high-performing tetraploid cultivar that has not yet been characterized in detail under drought.

Information for Kai was sourced from the New Zealand Plant Breeding and Research Association, National Variety Forage Trial data: http://www.nzpbra.org/forage-members/nfvt/P208BAL.pdf and Cropmark seeds http://www.cropmarkseeds.com/.

2.1.3 Tunisian population (A 14499)

This perennial ryegrass is a medium tillered diploid, with small leaves and a relatively prostrate growth habit in relation to the Samson and Kai populations. The accession was originally collected from an ungrazed roadside near the sea at the Turkish fort (Borj Massaoud) in Northern Tunisia in June 1994. The NPGS (National Plant Germplasm System) received the germplasm in October 1994 and a PI (plant identification) was assigned in 1997. The accession used in this experiment is an increase of the 1999 collection.

Plate 2-3 Tunisian population

The climate at the collection site is seasonally dry with a large proportion of the average annual rainfall of 1029mm falling over the winter months (Figure 2-1). The annual average temperature is 18.1°C, with summer temperatures commonly exceeding 30°C in June, July and August. Temperatures seldom fall below 15°C and average monthly temperatures are above 20°C for eight months of the year.

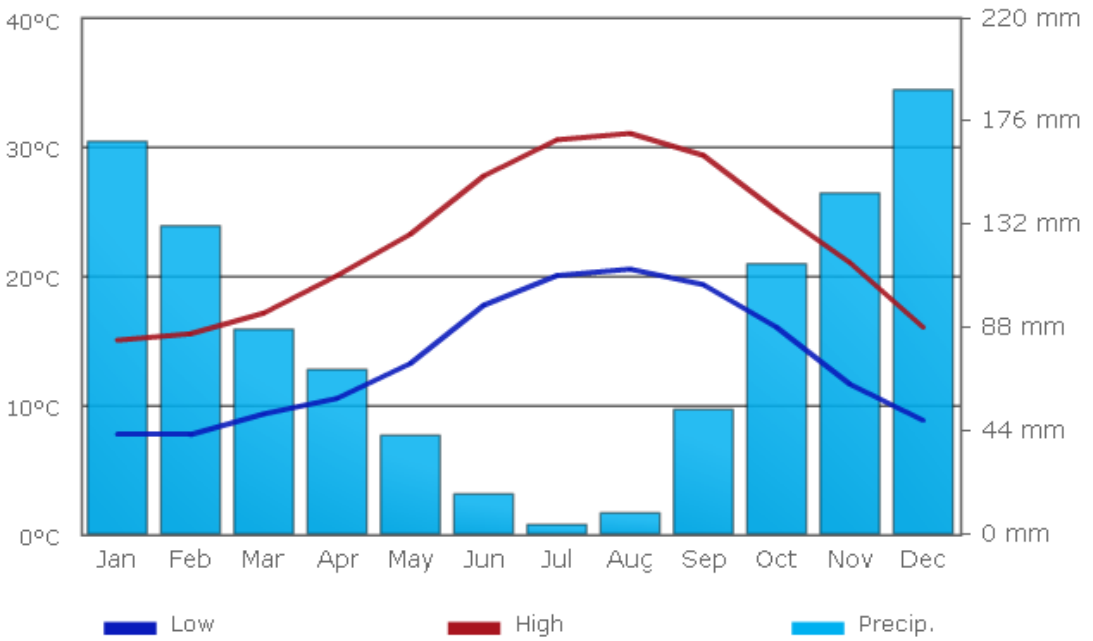

Figure 2-1 Climate data for Tabarka in Northern Tunisia. Source: http://www.climatedata.eu/climate.php?loc=tszzz0007&lang=en

The response of the Tunisian population to drought is of interest. This ecotype has likely been naturally exposed to periods of drought conditions continually throughout its life and has continued to grow and reproduce under stress. These conditions are similar to those experienced by plants in dryland New Zealand farms, but on a more extreme level. Through monitoring a plant ecotype that persists under periodic drought, important lessons may be learnt and applied for the improvement of perennial ryegrass.

2.1.4 Norwegian population (A 17183)

This diploid perennial ryegrass is medium tillered and small leaved, with a medium-erect growth habit. This accession was originally collected in Bryne, in South-West Norway and maintained by the Western PI station. The accession was then donated to the NPGS in Wales, by the Welsh plant breeding station in September 1991. The PI was assigned in 1994. This material was used in a study exploring the winter hardiness and turf quality of perennial ryegrass accessions (Hulke et al. 2007). This accession is an increase of the A 16719 collection from Norway.

The climate at the site of collection can be described as temperate however summers are relatively cool and wet in comparison with New Zealand summers. The average annual rainfall for the region is 1361mm with most rainfall (497mm) occurring between September and November. The driest months are typically from April to July, where the average rainfall is approximately 30mm per month. The average annual temperature for Bryne is 7.2°C, with temperatures reaching a maximum of about 16°C in June and July, and falling to an average of 2°C from December to March. Source: http://en.climate-data.org/location/9880/.

Plate 2-4 Norwegian population

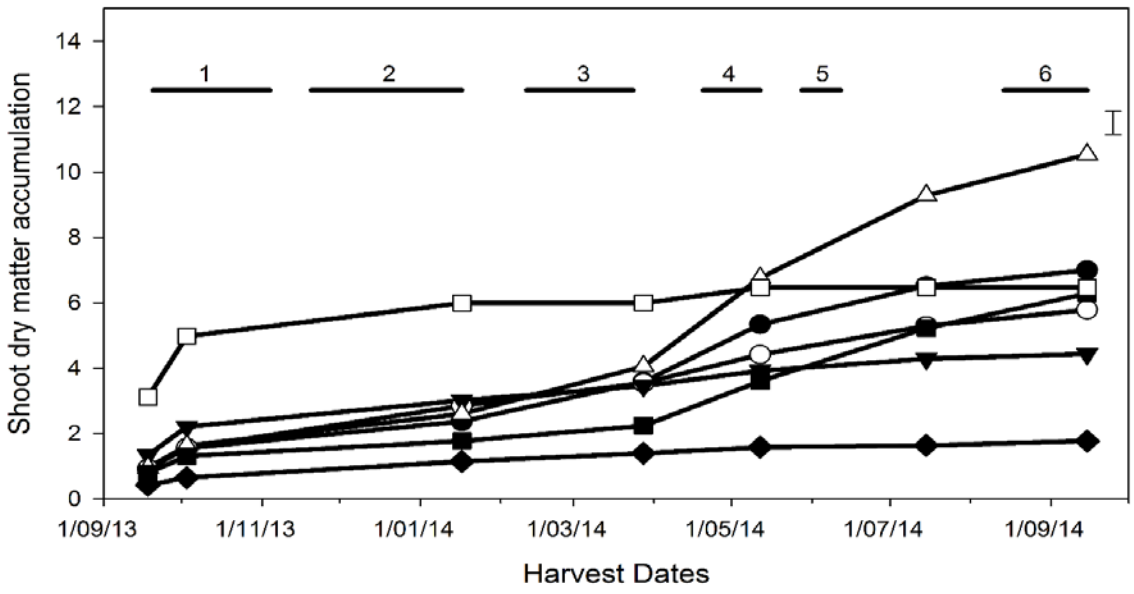

Figure 2-2 Shoot biomass from six *Lolium perenne* accessions and control (Impact) over seven drought cycles (Cyriac 2016). Accessions: Tunisian, 14499 (●); SW Europe, A14542 (○); Algeria, A15323 (▼); Norwegian, A17183 (△); Otago/Southland, A6889 (■); Russian, A77187 (□); Impact (◆). Drought cycles (——).

In previous work (Cyriac 2016) the Norwegian accession was shown to have an improved tolerance to drought in that it persisted over seven, drought cycles of 20 days in length and even increased shoot biomass, which was significantly larger than that of the commercial New Zealand cultivar Impact (Figure 2-2). While (Figure 2-2) shows that the Norwegian population only started to separate from the other accessions after three to four drought cycles, it is possible that it made physiological changes earlier, to better equip itself for dry conditions. The current work attempts to uncover some of those early responses. Considering its performance under drought (Cyriac 2016) despite its provenance from a climate that would seldomly facilitate drought conditions, the physiological responses of this germplasm are of additional interest.

Information for the Tunisian and Norwegian populations was retrieved from the National Plant Germplasm System, Germplasm Resource Information Network: http://www.ars-grin.gov/npgs/acc/acc_queries.html and Margot Forde Germplasm Centre, Palmerston North, New Zealand.

2.2 Drought

Definition of drought

Living organisms have two defining essences – a cellular organization and a requirement for liquid water (Jenks & Hasegawa 2005). Water makes up a significant proportion of the fresh weight of herbaceous plants where it acts as a solvent with unique biophysical and biochemical properties in significant plant processes and allows plant cells to maintain turgor.

Drought (or water deficit) may be defined as the point at which crop productivity becomes limited by sub-optimal rainfall (Hussain 2013). Drought is the single biggest threat to world food security and has been a catalyst of the great famines of the past (Farooq et al. 2009). Its severity is unpredictable as it depends on many factors such as frequency and distribution of rainfall, evaporative demands and the water holding capacity of a soil type (Wery et al. 1994). Agricultural regions affected by drought may lose 50% of their crop yield to drought (Jenks & Hasegawa 2005).

The frequency and severity of drought is likely to increase in New Zealand

The IPCC (International Panel for Climate Change) has forecasted changes in mean annual precipitation as well as the frequency of drought events in New Zealand (Hollis 2014). While these predictions are highly variable by region and season, the overall pattern is a reduction in rainfall in the north and the east of the North Island and pockets in the east of the South Island and increases in rainfall almost everywhere else, especially on the west coast of the South Island (Hollis 2014).

While annual rainfall is expected to decrease, the largest changes by the end of the century are likely to be for particular seasons (Mullan et al. 2005). In winter, reductions in precipitation are likely to be felt in Waikato, Gisborne, Hawkes Bay and the Canterbury plains whereas increases are expected for Nelson, the West coast, inland Canterbury, Otago and Southland. The reductions in winter rainfall for areas such as Canterbury and Hawkes Bay could pose a significant issue as agricultural systems rely on winter rainfall to ‘fill up’ the soil profile for use later on in summer.

Drought frequency and intensity is projected to increase in magnitude in most regions, except for Taranaki, Manawatu, the West Coast and Southland (Figure 2-3). The strongest increases are expected to be observed in the north and the east of the North Island as well as along the eastern side of the Southern Alps. There is also a projected increase in the number of dry days for much of the North Island, and for high altitude, inland regions of the South Island. The frequency of dry days is also expected on both coasts of the South Island (Mullan et al. 2005).

However agricultural drought is not a function of any single climatic factor, it arises from an extended deficit between rainfall and plant water use, to a point where soil moisture becomes depleted. A potential increase in the mid-range temperature in New Zealand of 0.2°C per decade is expected (Salinger 2003). This will likely exacerbate drought conditions by increasing potential evapotranspiration rates (PET) by around 5% by the year 2080. This translates to increases in PET of 50mm in Hawkes bay and 40mm in South Canterbury, with respective decreases in annual rainfall expected to be 125 and 55mm. The effect will be felt most heavily in the eastern regions of both islands, certainly increasing the likelihood of soil moisture deficits (Salinger 2003).

The significance of these projections for New Zealand agriculture are related to the farming systems in the affected regions. For the majority of the affected regions the main system is dryland farming. Dryland farming regions are generally defined within the farming community as those environments where summer evapotranspiration exceeds summer rainfall in the majority of years (Brown & Green 2003). In New Zealand, a total of 2,872, 295 hectares of land received 800mm of rainfall or less between 1950 and 1980; 10.7% of the total New Zealand land area. Dryland regions in New Zealand include parts of Northland, Waikato, Gisborne, Hawkes Bay, Wairarapa, Marlborough, Canterbury and Otago. Within these regions there are both extensive and intensive farmed areas, mainly limited to pastoral sheep, beef and deer grazing systems. The pastures that these farming systems have traditionally relied on perennial ryegrass, which is significantly constrained by hot, dry summers (Milne et al. 1993; Clark 2011).

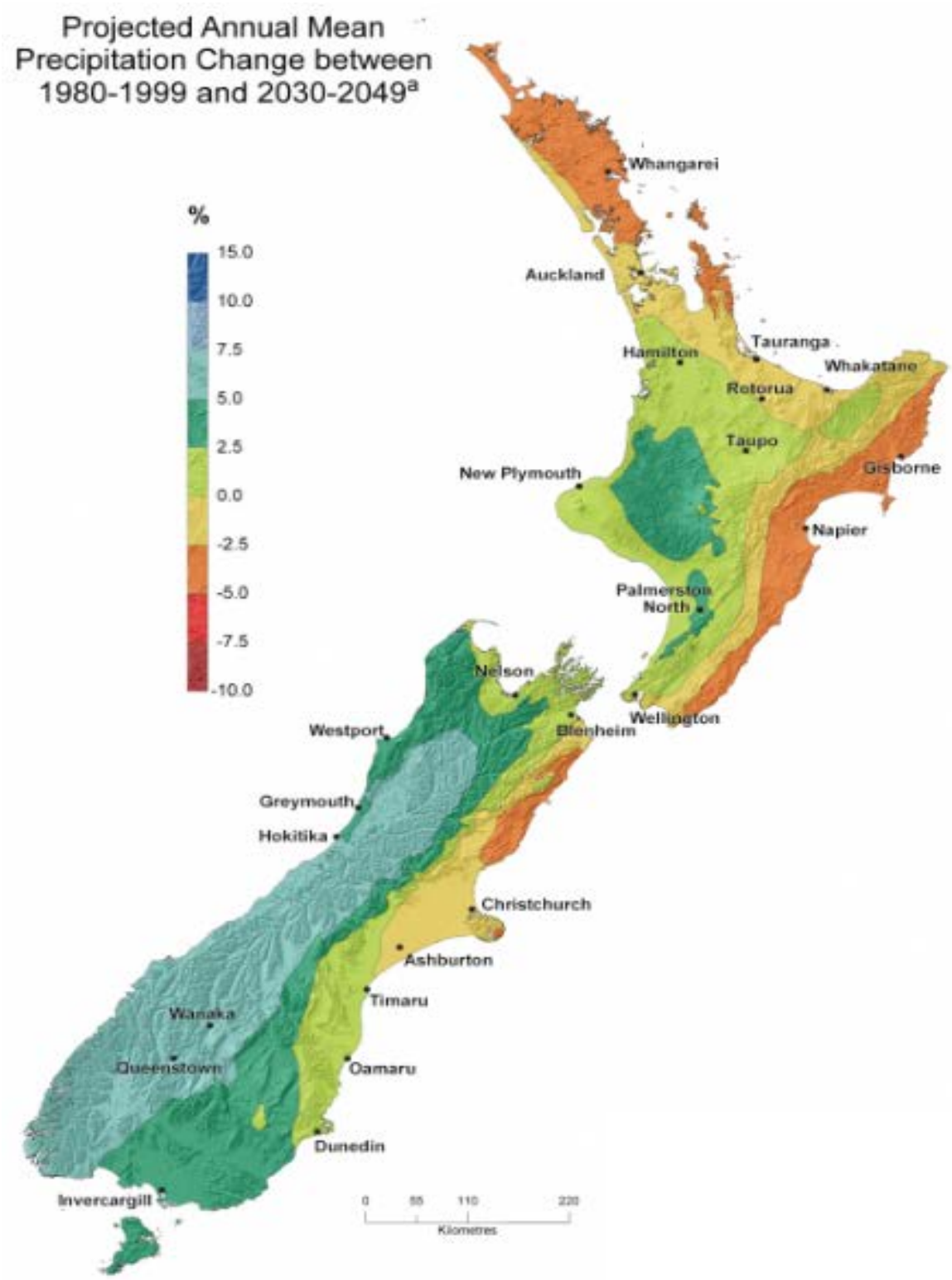

Figure 2-3: Projected regional changes in annual mean rainfall (%) relative to 1900: Average over 12 climate models. Source: https://www.niwa.co.nz/our-science/climate/information-and-resources/clivar/scenarios

2.3 General plant responses to drought

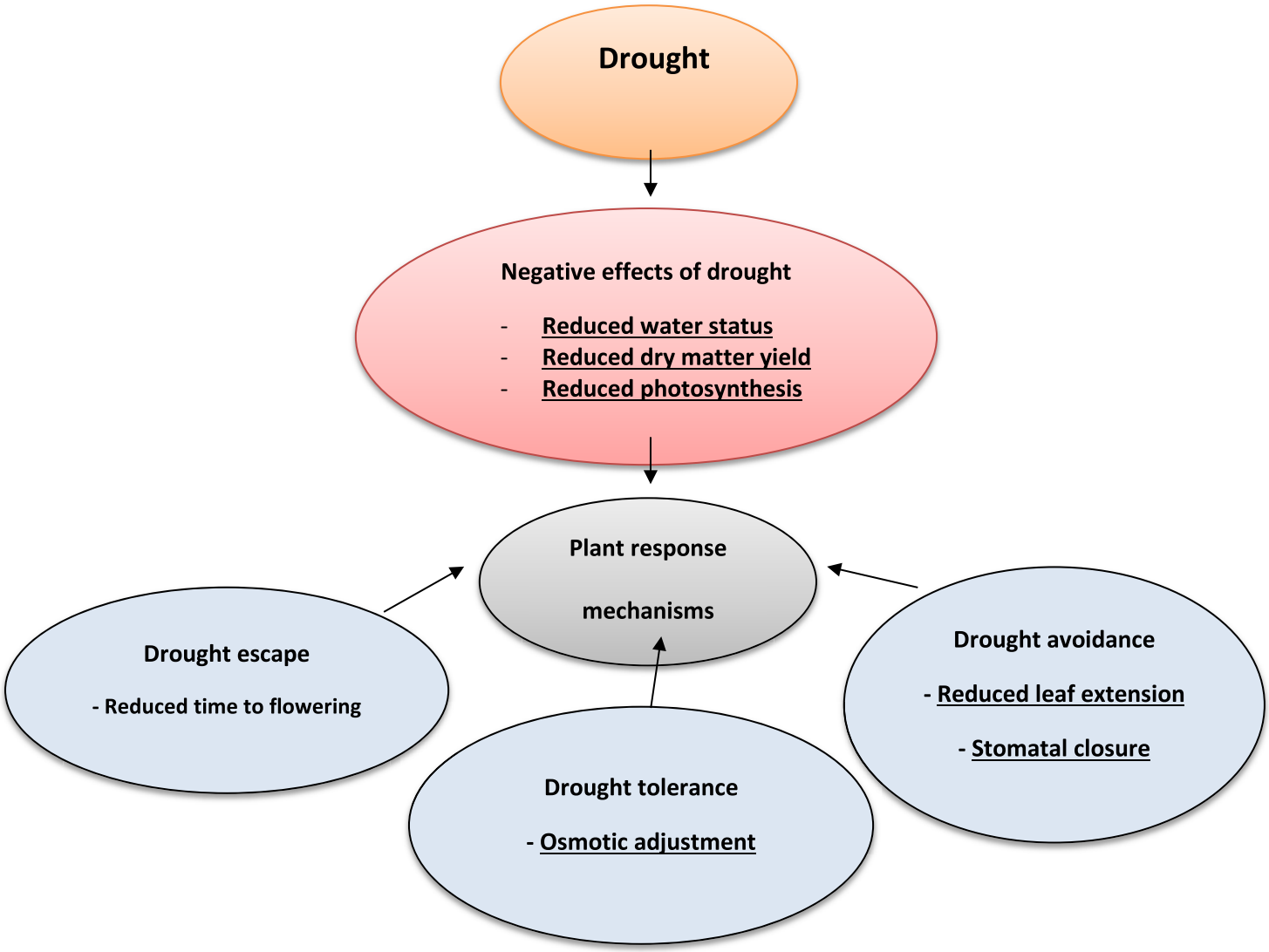

Figure 2-4 A schema of the effects that lead to plant responses upon exposure to drought. Effects underlined are referred to in this dissertation. Modified from Butler (2008).

The concept of drought stress is closely associated with a plants ability to cope with and overcome the drought, in order to eventually reproduce and continue to exist as a species. A plants' capacity to deal with drought denotes their level of drought tolerance or resistance. There are three means by which a plant can overcome drought, including; Escape, avoidance and tolerance.

Escaping drought refers to a plants ability to match their growth duration and length of life to periods of the year when drought may occur, for example flowering time is an important trait related to drought adaption, where a shortened life cycle, typical of annuals like subterranean clover (*Trifolium subterraneum*), may lead to drought escape (Araus et al. 2002). Drought avoidance represents a plant's ability to deal with a stress initially. In virtually all plants exposed

to an immediate water deficit, stomatal closure is the first response (Mansfield & Atkinson 1990). This will cause an instant decrease in water loss via transpiration, but also a decrease in the rate of photosynthesis because of a decreased acquisition of CO_2. Usually drought avoidance will provide the primary remedial action, but is generally unsustainable and will develop further into drought tolerance. Drought tolerance is directly related to the ability of a plant to persist in an adverse environment that is incorporated into their genetic material. For example, when plant cells begin to lose water to an extent where turgor pressure is diminished, plants may carry out osmotic adjustment, by accumulating solutes in cells to draw in water and re-establish normal turgidity.

Perennial ryegrass is a cool/moist season grass and is largely considered as drought sensitive (Glassey 2011; Matthew et al. 2012; He et al. 2013; Hussain 2013). However through the improvement of protective mechanisms it may be able to continue to persist in environments that are progressively becoming drier and make a valuable contribution to the feed supply of dry land farms in the future.

2.4 Plant responses to drought

2.4.1 Morphological

Above ground biomass accumulation

The total biomass yield of a pasture is dependent on the availability and interception of incident radiation and the ability of the species to convert energy into material that is economically useful (White & Hodgson 2011). If any one of these factors becomes limited, the dry matter yield of the pasture will decline. The yield of perennial ryegrass swards becomes limited during drought by way of a reduced interception of light through a decrease in leaf area and eventually a decrease in radiation use efficiency, should the drought become severe.

It is well known that perennial ryegrass is particularly susceptible to moisture deficit (Glassey 2011; White & Hodgson 2011; Matthew et al. 2012), which is likely the result of its relatively shallow root growth, resulting in a dependency for a continuous replenishment of water in the top of the soil profile (Mayfield & Neilson 1996). The inability of this root system to service the all-important above ground proportion during hot, dry summers leads to a reduction in yield.

The yield reduction of perennial ryegrass plants under drought has been quantified. Perennial ryegrass herbage production was significantly reduced in plants subjected to 3 months of drought (1800 kg/ha) in comparison with well-watered plants (5700 kg/ha) (Korte & Chu 1983). The reduction in herbage yield under drought was initially a result of decreased tiller weights as a

consequence of reduced leaf extension and appearance, but was also due to a decrease in tiller density later in the drought period. In other work (Barker et al. 1985), drought-exposed perennial ryegrass plots yielded only 8% of the herbage yield obtained by irrigated plots. The decrease in yield was attributed to lower tiller appearance rates, greater tiller death and decreased leaf appearance and extension rates.

Leaf extension rate

The primary reason for a reduction in dry matter production under drought, is the inhibition of leaf extension processes. Leaf extension occurs through cell division, cell differentiation and cell elongation, which all depend on the presence of water. Water provides the driving force for cell elongation through creating internal pressure in the cell known as turgor pressure. In cell elongation, parts of the cell wall are loosened and then forced out by the turgor pressure. The relationship between turgor pressure and cell elongation can be explained by equation 2-1 (Green et al. 1971).

Equation 2-1: Relationship between turgor pressure and growth;

$$G = m(P - Y)$$

Where G, the rate of cell elongation, is a function of cell wall extensibility (m), cell turgor pressure (P), and the minimum amount of turgor pressure required to force the cell wall outward (Y). Therefore, to maintain growth, plants must ensure they maintain a higher turgor pressure than what is required to expand the cell. However in a water deficit, water is drawn away from cells to an extent where the maintenance of cell turgor is unfeasible, and a subsequent inhibition of both cell elongation and thus lead extension, ensues.

The leaf extension of perennial ryegrass under drought has been analysed. In one study (Volaire et al. 1998), the leaf extension rate of perennial ryegrass plants declined to 50% of that of well-watered plants within four to six days after irrigation withdrawal, and 10% after 9-12 days. Also in other work (Norris & Thomas 1982) leaf extension rates of drought-exposed perennial ryegrass plants fell to less than 2mm day^{-1} after 14 days, where as well watered plants maintained leaf extension rates of 17mm day^{-1} over the same time frame.

A reduction in leaf extension rate can also serve as a drought avoidance mechanism. Large leaves provide an optimal surface for the production of photosynthates, but can reduce crop growth by providing a large surface area for the evaporation of water. Plants can deliberately reduce leaf area in response to a water deficit by reducing leaf cell division and expansion, as well as initiating leaf abscission and senescence of unnecessary leaves (Taiz et al. 2014). By reducing leaf area as

an early response to a water deficit through a reduction in leaf expansion, leaf rolling and leaf abscission, perennial ryegrass plants can keep dormant buds and underground organs alive for regeneration once soil moisture levels are replenished (Kemp & Culvenor 1994). In research by Korte and Chu (1983), perennial ryegrass plants exposed to water deficit had decreased tiller density and increased rate of leaf senescence, but the small leaves that remained were not particularly stressed physiologically. This then enabled rapid restoration of leaf area and tiller density, upon re-watering.

Root biomass

Research on New Zealand perennial ryegrass roots is generally limited, but interest in rooting systems is growing because of their potential influence in drought tolerance (Kemp & Culvenor 1994). The production of a large and fibrous root system is dependent on similar growth processes to that of the shoot (all above ground biomass). A relationship exists between the growth of roots and shoots that is set by the genetic potential of the plant; the shoot grows until the water uptake by the roots is limiting and the roots grow until their demand for photosynthates exceeds the supply from the shoots. There is evidence to suggest that this functional balance shifts following the onset of a water deficit.

When water becomes limiting, leaf extension is inhibited before photosynthetic activity is affected. During this phase there is reduced demand by shoots for Carbon and energy and thus a greater proportion of assimilates is available for supporting continued root growth (Taiz et al. 2014), for example; following the withholding of water, the root counts of perennial ryegrass plants appeared to increase for 1.5 to two months after the commencement of the drought (Wedderburn et al. 2010; Reid & Crush 2013). This increase in root growth (expressed as dry matter as opposed to root counts) may not exceed that observed in well-watered plants, but would likely result in an increase in the plant's root: shoot dry matter ratio. In situations where repeated root counts are not practical, i.e. a pot experiment, the root to shoot ratio provides an excellent measurement for observing this response. An increased capacity for this process may be important factor for the improved persistence in perennial ryegrass plants exposed to seasonal drought.

While this effect may be important for drought avoidance in short term droughts, it cannot be sustained without water. In the same work as above, the increased root growth was followed by a rapid decline in root counts in subsequent weeks. This suggests that this shift in partitioning of energy is only useful to a certain extent, as eventually with the absence of all water, the ability of

the plant to carry out photosynthesis will decrease, along with the root cells' ability to elongate. After this point root growth would become severely inhibited.

Additionally of interest is a trial conducted by (Crush et al. 2009) investigating perennial ryegrass root to shoot dry matter ratios involving 26 ecotypes from Europe and the Middle East, as well as number of bred populations. One of the findings was that wild types had higher root to shoot DM ratio than bred material. The effect may have been due to the continual selection for increased shoot biomass of the bred material. This may provide an advantage for the ecotypes over bred populations under drought.

2.4.2 Physiological

Plant water relations

Plant water relations are based on physics and chemistry where water will move from an area of high concentration to an area of lower concentration. In normal growing conditions, a concentration gradient exists between the interior of the plant and the surrounding atmosphere. While stomata are open and CO_2 is flowing in, water is drawn out of the leaf into the atmosphere and lost. The water loss from the leaf then creates a gradient which favours the movement of water from the xylem vessels to the leaf and from the soil into the xylem vessels originating in the roots. This process is known as transpiration and is the driving force for the movement of water through the soil-plant-atmosphere continuum.

Water potential

Water potential is defined as the chemical potential of the water divided by the partial molal volume of water (the volume of one mol of water). Water potential is thus a measure of the free energy of water per unit volume (J m^{-3}). These units are equivalent to pressure units such as the Pascal (Pa), and the unit of MPa is more commonly used to express water potential. Water potential is influenced by three factors, including concentration, pressure and gravity. Water potential is expressed as Ψ and plant water potential can be separated into solute potential Ψ_s, pressure potential Ψ_p and gravity Ψ_g.

Solute potential or osmotic potential represents the amount of dissolves solutes in the water. Solutes reduce the free-energy of water by diluting the water molecules. This can be described as entropy, where the ability of the water to 'do mechanical work' is reduced. Solute potential can be expressed as osmolality (moles of total dissolved solutes per volume of water [mol L^{-1}]) or in pascals. Pressure potential represents the effect of hydrostatic pressure on water potential. Positive pressure increases water potential and negative pressure decreases water potential. The

positive pressure that occurs inside plant cells is known as turgor pressure, which is essential to growth and stability. Pressure potential is commonly measured in plants as MPa. The gravitational pull of the earth causes water to move downward, that is unless it is opposed with an equal and opposite pull. It is largely based on the height of the plant and the density of water. However the gravitational component of water potential for considerations of water transport at the cellular level is insignificant and thus the water potential equation under such conditions can be simplified to:

$$\Psi = \Psi_s + \Psi_p$$

Water potential is often employed as a measure of plant water status, as with a decrease in water availability there is usually a decrease in water potential. Figure 2.4 shows the sensitivity of some important plant physiological processes to a decreasing water potential.

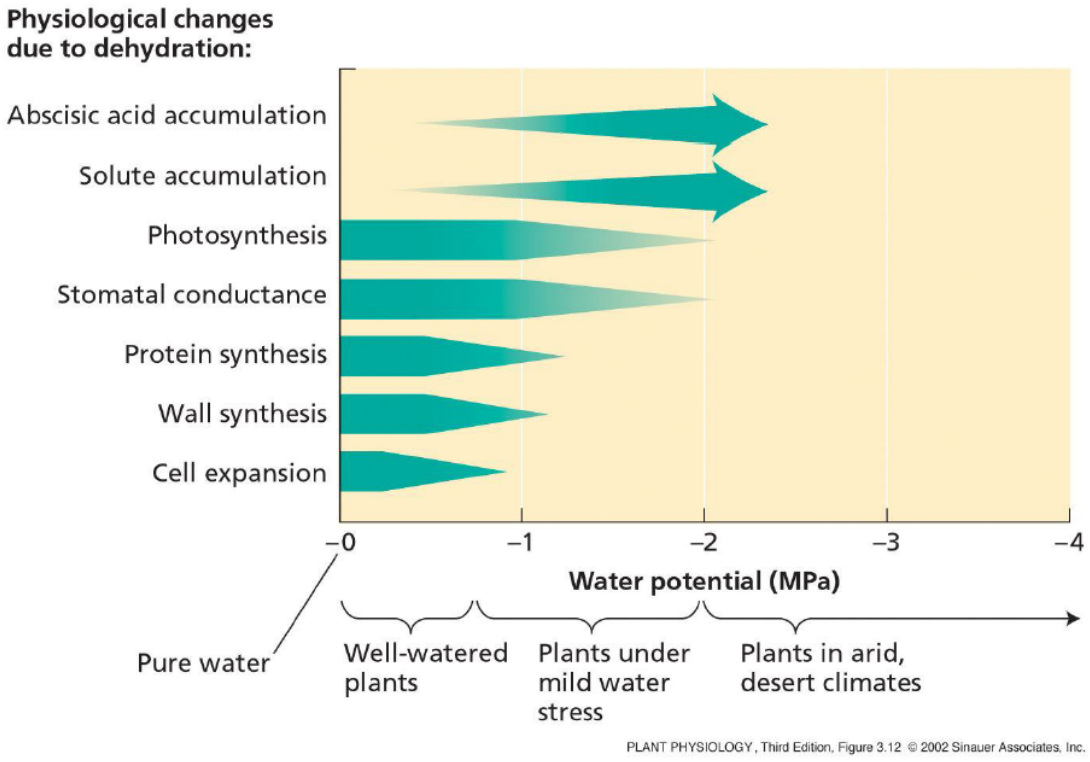

Figure 2-5: Sensitivity of various physiological processes to changes in water potential. The thickness of the arrow corresponds to the extent of the process (Taiz et al. 2014).

The sensitivity of important plant physiological processes to a water deficit differs. The rate of cell expansion, related to leaf expansion, is extremely sensitive to a decrease in water potential, whereas photosynthesis is affected only at a greater water deficit.

Relative water content

Relative water content (RWC) is an alternative, morphological measure of plant water status. It is the water content of plant cells compared to the water content of plant cells at their maximum water holding capacity (full turgidity) (Pask et al. 2011), calculated as follows:

Equation 2-2 Formula for RWC:

$$\mathrm{RWC} = \frac{\mathrm{FW} - \mathrm{DW}}{\mathrm{TW} - \mathrm{DW}}$$

where FW is the fresh weight at harvest, DW is dry weight after drying to constant weight in an oven and TW is turgid weight (where the leaf is immersed in water so that it can gain full turgidity) (Ehlers & Goss 2016). When the RWC of leaves is measured, it can provide an indication for the severity of a water deficit and the degree of stress that the plant is under. Wilson (1975) found that the rate of leaf extension of perennial ryegrass leaves started to decline when leaf RWC fell below 88%. Plants that can maintain a high leaf RWC under water deficit, i.e. maintain leaf turgidity with a lesser amount of water available, are likely to have improved performance under drought conditions (Pask et al. 2011).

Osmotic adjustment

With the onset of a water deficit, the water potential in the rhizosphere (just outside the root hairs) is reduced, resulting in a gradient that favours the movement of water from plant roots, back into the soil. Plants must decrease their internal water potential further to continue to take up water. By accumulating solutes, plants can drive the osmotic potential of intracellular fluid down and so reduce the water potential inside the plant, to draw water into the roots. This process is known as osmotic adjustment and is one of the most important protective physiological responses of plants to drought (Taiz et al. 2014). This adjustment can be typically attributed to the accumulation of common solutes within the cytoplasm, including sugars, organic acids, amino acids (proline and aspartic acid) and inorganic ions, especially potassium (K^+). Osmotic adjustment helps to maintain cell water balance and minimizes other harmful effects of drought (Farooq et al. 2009).

In wheat cultivars, an increased capacity for osmotic adjustment has been labelled as an important prerequisite for drought tolerance (Akıncı & Lösel 2012). Osmotic adjustment is a common protective response to water deficit in many cool season grasses (Frank et al. 1996). In other work (Thomas 1986) perennial ryegrass subjected to a slowly developing drought adjusted osmotically by as much as 0.8 MPa (mega pascals), more than cocksfoot (*Dactylis glomerata*) and

italian ryegrass (*Lolium multiflorum*) under the same regime. This indicates that osmotic adjustment may be a key response mechanism of perennial ryegrass plants subjected to drought. Perennial ryegrass has also been shown to accumulate up to 12 times the amount of proline under drought conditions compared to that when irrigated (Volaire et al. 1998), although the contribution of proline to osmotic adjustment in grasses is somewhat contested (Ashraf & Foolad 2007; Hahn et al. 2008).

Methods for the appropriate measurement of solute potential and ultimately osmotic adjustment were compared by Babu et al. (1999). There are four different methods to date:

1.) (Morgan 1992);

The RWC and Ψ_s are obtained from consecutive measurements during a drought cycle. Osmotic adjustment is calculated as the difference between the actual solute potential of the drought-exposed leaves and the proportion of Ψ_s that can be ascribed to the mere water loss of water from the leaves at a given RWC.

2.) (Wilson et al. 1979); the osmotic adjustment is estimated from the difference in Ψ_s between well-watered and drought stress replicates, but both Ψ_s's are calculated at a well-watered state (Ψ_s100). $\Psi_s100 = \Psi_s$ [(RWC-B)/ (100-B)}, where B refers to a correction for water in the apoplast tissue. Different plant species have different values of B.

3.) (Blum 1989); the osmotic adjustment is estimated from the difference of the Ψ_s between well-watered plants and re-hydrated drought-stress plants. Stressed plants are irrigated in the evening and sampled the next morning for the Ψ_s determination.

4.) (Morgan 1995); By regressing the RWC of drought exposed leaves against the proportion of Ψ_s ascribed to cell dehydration, a higher RWC for at any given Ψ_s indicates higher osmotic adjustment in those plants.

Babu et al. (1999) compared these four methods in 12 rice cultivars and found that the mean osmotic adjustment over the 12 cultivars was 0.89 MPa, 0.51 MPa and 0.71 MPa for methods one, two and three respectively. Simple correlation coefficients of methods two, three and four with method one were 0.54, 0.76 and 0.87 respectively. The coefficient of variation as an indicator of error was 47% in method one, 31% in method two, 21% in method three and 24% in method four. Method two and three required less labour and plant material in comparison with methods one and four, with the conclusion that method three can be considered as the replacement for method one, which is quick, accurate and economic.

Stomatal closure

Plants possess specialised epidermal guard cells on leaf surfaces, which act as hydraulically driven valves, forming stomatal pores (Roelfsema & Hedrich 2005). These cells control gas exchange and water loss from the internal leaf structures responsible for photosynthesis, by regulating stomatal aperture. Stomatal aperture is controlled by the turgidity of guard cells. As the guard cells become turgid, the space between them widens, allowing more flow of water and/or gas in and out of the leaf. As the guard cells lose turgidity and become flaccid, the space between them is reduced, creating a greater resistance to molecules trying to pass through.

Stomatal closure is the first response to a water deficit, to reduce the rate of transpiration and thereby protect the plant from excessive water loss (Chaves et al. 2003).Environmental factors that influence stomatal closure include the relative humidity of the surrounding air (a low relative humidity will favour water loss as water vapour is drawn down the concentration gradient), light quality and CO_2 concentration (Farquhar 1978; Taiz et al. 2014). Plant hormones also play a role in stomatal closure. Water deficit induces the accumulation of ABA (abscisic acid) in the leaves. ABA promotes stomatal closure through the polarization of plasma membranes which allow for the efflux of K^+ (Potassium ions) from the guard cells, thus decreasing extracellular water outside the cell. This effect draws water from the guard cells and causes them to become flaccid, resulting in the closure of stomata.

The process of transpiration, acts as mechanism of drawing heat away from the plant, allowing the plant to maintain an optimal physiological temperature (Jiang & Huang 2001). In drought stressed plants, a higher leaf temperature is indicative of stomatal closure, in that the primary cooling process has been inhibited. (Jiang et al. 2009) endorsed the measurement of the difference between canopy temperature and air temperature for effectively predicting leaf water content of perennial ryegrass under water deficit. The measurement of stomatal closure through the use of an infra-red gun to monitor the canopy temperature of plants is a proxy to more common methods of measuring stomatal conductance such as a porometer, or a gas exchange system such as a LICOR 6400 (Idso & Baker 1967; Hatfield 1990).

The argument is ongoing as to the relationship between leaf temperature and water use efficiency, and whether a drought-tolerant plant would have warmer or cooler leaves than the population average. It was proposed by (Blum 2009) that breeding for decreased stomatal conductance in cereals (characterised by warmer leaves), may result in decreased yields through a reduced extraction of soil moisture during crop growth. This would suggest that a plant with cooler leaves likely has a greater ability to extract soil moisture. However another strategy of

interest is around plants that can maintain leaf extension processes at a comparatively lower water use. These plants would give farmers more dry matter production under drought, compared with a plant that uses soil moisture quickly for the same dry matter production, but the plant depleting soil moisture more slowly would be expected to exhibit warmer leaves (Hussain 2013).

The regulation of stomatal closure by drought stressed plants is important for water conservation and may have a significant bearing on their water use efficiency (Davies et al. 2002).

Changes to photosynthetic capacity

As mentioned above, the first response of all plants to a rapid on-set of water deficit is the closure of stomata, to prevent water loss via transpiration. While this usually increases water use efficiency, it results in a decreased inflow of CO_2, potentially not only resulting in a decrease in photosynthesis by a lack of resources, but by an oversupply of electrons for the formation of reactive oxygen species (Farooq et al. 2009).

Extreme water deficit can thus result in damaged photosynthesis apparatus, as a result of oxidative damage.

In a study by Fu and Huang (2001) the chlorophyll content of tall fescue and Kentucky blue grass (*Poa pratensis* L.) declined after a 28 day drying treatment, with chlorophyll a exhibiting a larger reduction than that of chlorophyll b. Detrimental effects on chloroplast biochemistry measured by chlorophyll fluorescence, occurred when RWC dropped by 60%. It was proposed that the reduction in chlorophyll could be related to photo-oxidation resulting from oxidative stress. (Fu & Huang 2001) also found that an irreversible decrease in the photosynthetic capacity of a plant occurs when the RWC is reduced by more than 30%, as a result of cell death following membrane damage in the chloroplasts.

Leaf chlorophyll concentration is often correlated with plant metabolic activity, including photosynthetic capacity and Rubisco (Ribulose-1, 5-bisphosphate carboxylase) activity (Wood et al. 1993; Nageswara et al. 2001). The chlorophyll molecule contains four nitrogen atoms and extractable chlorophyll in leaves is positively correlated to leaf nitrogen concentration (Takebe et al. 1990). In a study on wheat (*Triticum aestivum* L.) and (Seemann et al. 1987) in common bean (*Phaseolus vulgaris* L.) (Evans 1983) the rate of CO_2 assimilation was directly related to leaf nitrogen content irrespective of other treatments, season or leaf age. Both chlorophyll content and rubisco activity were proportional to leaf nitrogen content in those experiments.

Leaf chlorophyll can be estimated with a SPAD (Soil Plant Analysis Development) meter. This device determines leaf greenness by utilizing the light attenuation difference between 650 and 940 nm (Wood et al. 1993). This is a much more efficient and less costly measure of leaf chlorophyll than the traditional colorimetric method that measures extractable chlorophyll, which is time consuming, destructive and requires sophisticated laboratory equipment. Importantly, for most studies, the relationship between extractable chlorophyll and chlorophyll meter readings has been linear (Wood et al. 1993).

For the purpose of this experiment, the measurement of leaf chlorophyll concentration via a SPAD meter will also be used in the current study as an indicator of plant photosynthetic capacity.

2.5 Role of endophytes in conferring drought tolerance in perennial ryegrass

Endophytes are naturally occurring fungi that complete their life cycle within grasses, including perennial ryegrass and tall fescue. They grow between the cells of the host plant, and in return confer protection against insects, overgrazing and potentially the harmful effects of a water deficit. While endophyte protection of tall fescue by *Neotyphodium coenophialum* under drought is widely accepted (Cheplick 2004; Tozer et al. 2007), the effect of *Neotyphodium lolii* on the drought tolerance of perennial ryegrass is generally contested (Saikkonen et al. 1998). (Cheplick 2004) indicated that under drought, perennial ryegrass infected plants were detrimentally affected by endophyte and that the relationship only benefited the endophyte. perennial ryegrass plants hosting *N. lolii* had a lower number of tilers, a smaller leaf area and less biomass than perennial ryegrass plants not hosting endophyte after three sequential droughts of 11-14 days. The fungal hyphae had survived the drought phase and were abundant in endophyte positive perennial ryegrass replicates.

Conversely (Hahn et al. 2008) reported that *N. lolii* delivers protection to perennial ryegrass under water deficit, however the relationship was not clear between the presence of endophyte and degree of drought tolerance. They found that plants hosting endophyte did not accumulate biomass to the same extent as plants not hosting endophyte, but took up less water and had a higher RWC than the plants without endophyte. This growth cessation could potentially be regarded as a proactive adaption to stress, rather than a response to the harmful effects caused by drought stress. This would protect the plant from any subsequent injury.

(Zhou 2013) ascertained that the presence of *N. Lolii* benefited perennial ryegrass plants under water deficit. Their results suggested that the endophyte increased drought tolerance in the grass through: (i) reinforcing chloroplast protection by increasing chlorophyll and chloroplast biosynthesis and ROS (reactive oxygen species) scavenging capability and (ii) by reducing water

loss by enhancing osmotic adjustment through increased glucose, fructose and maltose accumulation. These effects allowed endophyte-infected plants to maintain intracellular water balance, maintain a higher rate of photosynthesis and accumulate more biomass than plants without endophyte. Similarly (Hesse et al. 2003) found that infection with endophyte is beneficial for perennial ryegrass. Endophytes induced a rapid re-establishment of roots in the recovery phase after a period of drought, which is likely to be important for the re-establishment of tillers and leaf area expansion as shoot growth resumes.

Additionally (Eerens et al. 1998) suggested that the impact of the endophyte on a perennial ryegrass based pasture is largely dependent on environmental conditions, i.e. the severity of a water deficit. (Hesse et al. 2003) also proposed that the environmental conditions in the original habitat of the perennial ryegrass genotypes may influence the symbiotic relationship between fungus and plant.

Recognising the potential impact of endophyte presence on the effects of drought and drought responses by perennial ryegrass plants, it is sensible to screen for endophyte presence in the current study.

2.6 Conclusions of literature review

To conclude this literature review, there is increasing concern for the production and persistence of perennial ryegrass based pastures in dryland farms, especially in northern and eastern regions of New Zealand. This is mainly due to the projected increases in drought frequency, as perennial ryegrass is highly sensitive to soil moisture deficit. Potentially suitable perennial ryegrass germplasm for increased production and persistence in drier areas may include perennial ryegrass ecotypes.

There is extensive literature on physiological protective response mechanisms to drought in plants and some studies are also quantifying the impact of drought on perennial ryegrass morphology. However evidence is lacking for the physiological responses of perennial ryegrass to drought and more importantly the responses of perennial ryegrass to drought in New Zealand.

The aim of this dissertation is to compare the morphological and physiological responses in bred perennial ryegrass populations and ecotypes under a controlled drought.

3 Materials and methods

Experimental design and conditions

The experiment was carried out in a in a glass house at Lincoln University, Canterbury, New Zealand from April to September 2016.

The trial was arranged in a 2 × 4 factorial split plot design, with 12 blocks/reps and two water treatments. In each block/rep, the four perennial ryegrass populations, were either exposed to a drought treatment or a well-watered treatment. Each of the sub plots represented a water treatment and contained one pot of each perennial ryegrass population. There was a total of 96 pots with five plants arranged evenly in each pot.

Plate 3-1 Layout of experiment in the glasshouse.

Seeds of four perennial ryegrass populations; Samson, Kai, Tunisia (A 14499) and Norway (A17183) were sourced from the AgResearch Margot Forde Germplasm Centre in Palmerston North New Zealand. Kai, Tunisia and Norway were sown on 27th of April, but the sowing of Samson seeds was delayed by 10 days due to unforeseen reasons. Seeds were planted in germination trays (200 of each population) containing a sowing mix of peat (30L), sterilised pumice (20L), Osmocote Exact 3-4 month slow release fertiliser (100g) (16-5-9.2-0 + 1.8%Mg), dolomite lime (200g) and Hydraflo wetting agent (50g). Once seedlings had reached the 3-4 tiller stage, the healthiest plant were transplanted into the larger, 7.5 Litre experimental pots.

The soil in the pots was comprised of 75% Wakanui silt loam (sourced from the Horticultural Research Area at Lincoln University) and 25% mortar sand (0.1-3mm). Also 2g/L Osmocote Exact 3-4 month slow release fertiliser and 1g/L Hydraflo were added to the mixture. Soil was poured into the pots so that the level was between 2-4cm below the rim. The soil was not compacted during this process. At transplantation, pots were watered and then five small holes (5cm in diameter) were made in the soil surface for the perennial ryegrass seedlings to be placed in. At this stage the excess soil from the holes increased the soil height within the pot so that the soil surface and base of grass tillers were no lower than 2cm from the top of the pot rim.

Experimental conditions

Over the first drought cycle temperatures ranged between 14°C and up to 26°C, with an average of 18°C during the day and 16°C during the night. Relative humidity ranged between 35% and 70% and averaged 55%. PAR (incident photosynthetically active radiation) ranged between 100 and 500 mmol $m^{2\,-1}$ and averaged 320 mmol $m^{2\,-1}$. During the second drought cycle, as day length began to increase, temperatures ranged between 14°C and 27°C and averaged 20°C during the day and 17°C during the night. Relative humidity ranged between 30% and 70% and averaged 55%. PAR levels were comparable to the first drought cycle.

Time scale

Table 3-1 Overview of experiment time scale

Event	Date
Seeds sown	27/04/2016
Seedlings transplanted	27/05/2016
Plants trimmed, first drought commenced	07/07/2016
Leaf dry matter cut, end of first drought	04/08/2016
Second drought commenced	04/08/2016
End of second drought, final harvest	02/09/2016

Water treatments

There were two treatments applied in each block; well-watered and drought. Well-watered pots were watered continuously throughout the trial. Pots were weighed every two days over the drought cycle to determine soil moisture content and ensure that this would remain around 20%. The drought-exposed pots were not watered at all over the two 28 day drought cycles. However after 28 days of drought, water-stressed pots were returned to field capacity gradually over the course of the 29th day. The following day then served as the harvest day and the commencement of the subsequent drought cycle.

Soil moisture

Soil moisture percentage was used as a measure for the effectiveness of our water treatments and included all 12 replicates. The original strategy for determining soil moisture was to use a TDR (Time Domain Reflectometer), where the connecter head with two metal prongs would be inserted into the soil to a depth of 15cm. However the soil in the drought pots became so firm that the prongs were not able to slide into the soil without excessive force, so the method was abandoned.

Instead the soil moisture percentage was determined gravimetrically, by determining the weights of the soil at fresh weight (FW) and comparing them to their dry weight. The FW was the weight of the soil at the highest point of the drought, i.e. after 28 days of drought, before the re-watering of the pots for the plant harvests. The soil dry weight (DW) was achieved by taking three pots from the well-watered treatment and three drought pots, post-harvest, and drying down the soil for 10 days at 60°C.

Equation 3-1; calculation of soil moisture percentage was as follows:

$$\left(100 \times \frac{FW}{DW}\right) - 100$$

Biomass accumulation

Dry matter harvests were completed before the commencement of the first drought cycle and at the end of each of the two drought cycles and included 6 replicates. All pots were watered to field capacity in the evening before harvest and cuts for leaf dry matter determination were carried out early the next morning. These cuts were taken at pot height (2-4cm plant height) and included all material above that height in each pot. Leaf dry matter samples were weighed and then oven dried at 60°C for at least 48 hours before determining the dry matter per pot with a two point balance.

At the final harvest, leaf dry matter, stubble dry matter and root dry matter were determined. Leaf dry matter per pot was determined as above for all pots. Following this, the stubble (above ground biomass below the height of the rim of the pot) was removed so that the only plant material remaining in the pot was below the soil surface. Once four complete blocks of pots were harvested down to soil level, the soil was washed away from the roots carefully in a root washing facility. Once all leaf, stubble and root material was sampled and bagged, samples were placed in the ovens and dried for at least 48 hours at 60°C. Following this, dry matter was determined with a two point balance. All biomass data is expressed as grams per pot. Total above ground biomass was calculated as the sum of leaf dry matter and stubble dry matter. The root to shoot ratio was determined by dividing the root dry matter weight by the shoot dry matter weight.

Leaf extension

Leaf extension measurements were carried out during the course of the two water drought cycles. Approximately one week after the commencement of each drought cycle, two tillers, each with a new leaf, per pot, were marked with a short piece of insulated wire. The first new leaf was measured from base to tip every three to four days until that particular leaf stopped growing. At that point, the next leaf on that tiller was measured, and so on. Leaf extension rates were calculated by dividing the sum of all leaf growth for one tiller, by the number of days of growth. These measurements were conducted in six replicates.

Canopy temperature

Canopy temperature was determined at the end of each drought cycle, prior to re-watering and included six replicates. It was achieved by holding an infrared thermometer 10cm above the canopy until the temperature reading was steady. This was used as a proxy measurement for stomatal aperture, as other methods such as a LICOR 6400 gas exchange system or a porometer were not practical because drought leaves were too thin and fragile. A higher canopy temperature indicates that stomata are less open to release heat energy via transpired water vapour.

SPAD

This measurement was included as a quick and accurate estimation of the relative chlorophyll concentration of leaves and included six replicates. Two randomly selected, young and growing leaves were chosen for the measurement in each pot. This measurement can also be used as an estimate of the photosynthetic capacity of leaves (Wood et al. 1993).

Relative water content

The relative water content of the drought-exposed and well-watered leaves of six replicates was measured at the end of the second drought cycle, before the re-watering of pots for the main harvest (harvest two). Two fully expanded, green leaves were taken from each drought pot and weighed with a four point balance to determine fresh weight (FW). The leaves were then placed into separate petri dishes and fully submerged in water. The petri dishes were subsequently placed in darkness at 4˚C for 24 hours so that leaves can take in as much water as possible. Following this, leaves were removed from the petri dishes and excess water removed carefully with paper towels. These leaves were then weighed again using a four point balance for the determination of leaf turgid weight (TW). Leaves were then placed into labelled bags and dried in an oven at 60°C for 48 hours. Following this, leaves were weighed again with a four point balance to determine the dry weight of the leaves.

Equation 2-2 calculation of RWC was as follows:

$$\mathrm{RWC} = \frac{\mathrm{FW} - \mathrm{DW}}{\mathrm{TW} - \mathrm{DW}}$$

Osmotic potential

The method used in this experiment is similar to that used by (Blum 1989). Stressed plants were watered in the evening and sampled the next morning for osmotic potential (OP) determination. This took place during the main harvest (after two drought cycles) and included six replicates. Fully expanded leaves were placed into 1.5 mL Eppendorf tubes containing a sieve that would be used to separate cell cytoplasm and fibre following the freeze-thaw phase. Following the sampling of leaf samples from both well-watered and drought-stressed plants, samples were frozen at -18°C overnight. The following day samples were taken out of the freezer to thaw. All sample tubes were then placed in liquid nitrogen for about 10 seconds and then left to thaw again to ensure the complete rupture of cells. Thawed samples were then centrifuged for 5 to 10 minutes depending on the amount of cell sap extracted from the leaf sample. The sap was then pipetted into an osmometer (Wescor, Logan, Utah) and the osmotic potential of the samples determined. Osmotic adjustment was calculated from the difference in the OP between well-watered plants and rehydrated drought-stress plants. Babu et al. (1999) found this method to be the most economical, efficient and accurate of the various methods used to determine osmotic adjustment.

Endophyte screening

The screening for endophyte in our perennial ryegrass populations was carried out using the immuno-detection method as described in (Simpson et al. 2012).

3.1 Statistical analysis

Analysis of the main effects and of their interactions were performed with the GENSTAT software (16th Ed, VSN International Ltd) General Analysis of Variance procedure using a split-plot model. Where appropriate, the $LSD_{P<0.05}$ was used to further explore interactions. Residual plots for ANOVA were checked for homogeneity of variance to determine whether transformation of the data was necessary. Significance of correlations was tested with regression analysis in Microsoft Excel.

Plate 3-2 Block 6 near the end of the second drought cycle.

4 Results

4.1 Main effects and interactions

The drought treatment reduced most of the morphological and physiological traits measured here, and increased the root to shoot DM ratio, canopy temperature and leaf chlorophyll concentration (Table 4-1). Population differences were largely dependent on the impact of the treatment.

Table 4-1 Table of statistical significance for all traits, with water treatment effect, population and water treatment × population effects following the second drought cycle (unless otherwise specified).

Trait	Treatment	Population	Treatment x Population
Soil moisture	*** DR↓	ns	ns
Shoot DM (dry matter)	*** DR↓	***	*
Leaf DM Harvest 1	*** DR↓	*	**
Leaf DM Harvest 2	*** DR↓	**	**
Leaf extension Cycle 1	*** DR ↓	*	**
Leaf extension Cycle 2	** DR ↓	+	**
Stubble DM	*** DR↓	***	***
Root Biomass	*** DR↓	ns	ns
Root DM: Shoot DM	* DR↑	*	*
RWC	*** DR↓	ns	ns
Adj. Solute Potential	*** DR↓	**	*
Canopy Temperature	*** DR↑	ns	ns
SPAD	** DR↑	ns	ns

Water treatments: **DR** drought; **WW** well-watered. Statistical significances: *** P < 0.001 = “very highly significant”; ** P < 0.01 = “highly significant”; * P < 0.05 = “significant”; + P < 0.10 = “marginal”. Direction of arrow following the ‘DR’ indicates drought-induced change in that particular trait.

4.1.1 Soil moisture

When comparing the two drought cycles, there was no significant harvest x treatment x population interaction for soil moisture, showing that for this attribute drought effects did not differ across the four populations over time. The drought treatment reduced the soil moisture content from 17.9-20.3% in well-watered pots to 5.2-7.8% in drought-exposed pots ($P < 0.001$; Figure 4-1). A significant treatment x population interaction showed that compared to the other populations (-66% on average), soil moisture levels in the Tunisian ecotype were reduced to a lesser degree in response to drought (-53%, $P < 0.05$). The drought effect was similar between the two drought cycles, reducing soil moisture levels by an average 63% ($P < 0.05$).

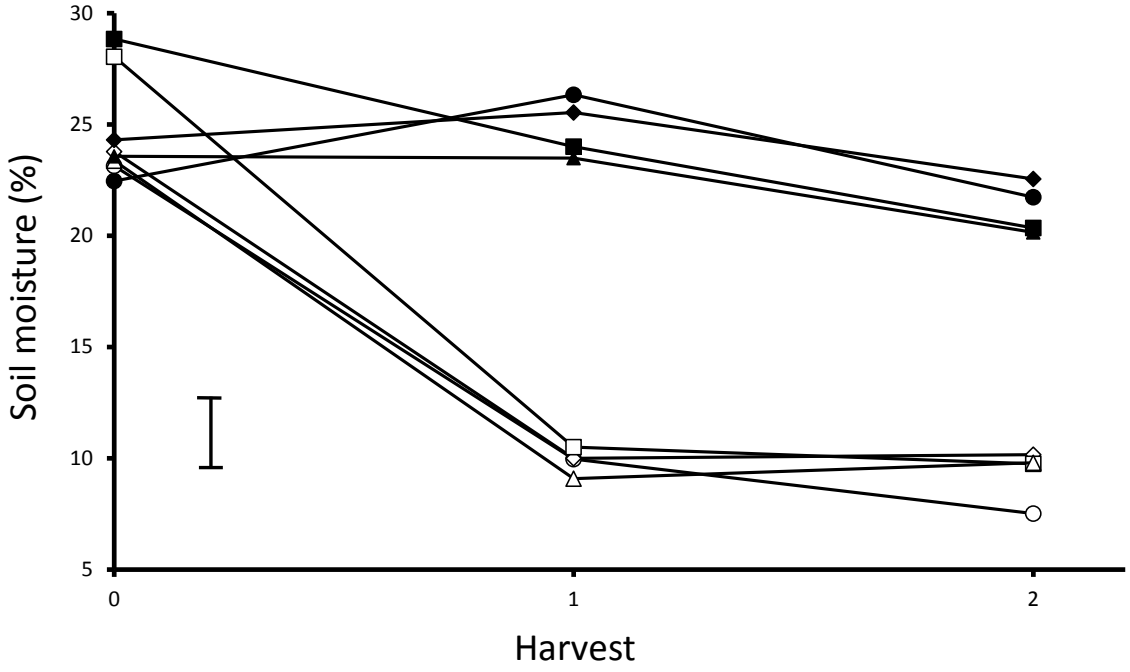

Figure 4-1 Soil Moisture content (%) of four perennial ryegrass populations prior to the first drought cycle, following the first drought cycle and following the second drought cycle. *Lolium perenne* L. populations: Kai WW (●), DR (○);Samson WW (■), DR (□); Norwegian WW (◆), DR (◇); Tunisian WW (▲), DR (△).The bar is the LSD $_{P<0.05}$ for the harvest x treatment x population interaction.

4.1.2 Shoot biomass accumulation

The total shoot DM (dry matter) accumulation for the well-watered Kai plants at the main harvest was 43% greater than the mean of the other well-watered populations (Figure 4-2). The drought treatment caused a reduction in total shoot biomass across all populations (Table 4-1). In the drought treatment, all populations had comparable shoot DM weights. However the shoot biomass of the Norwegian population was reduced least by drought (-58%) and most in the Kai cultivar (-69%).

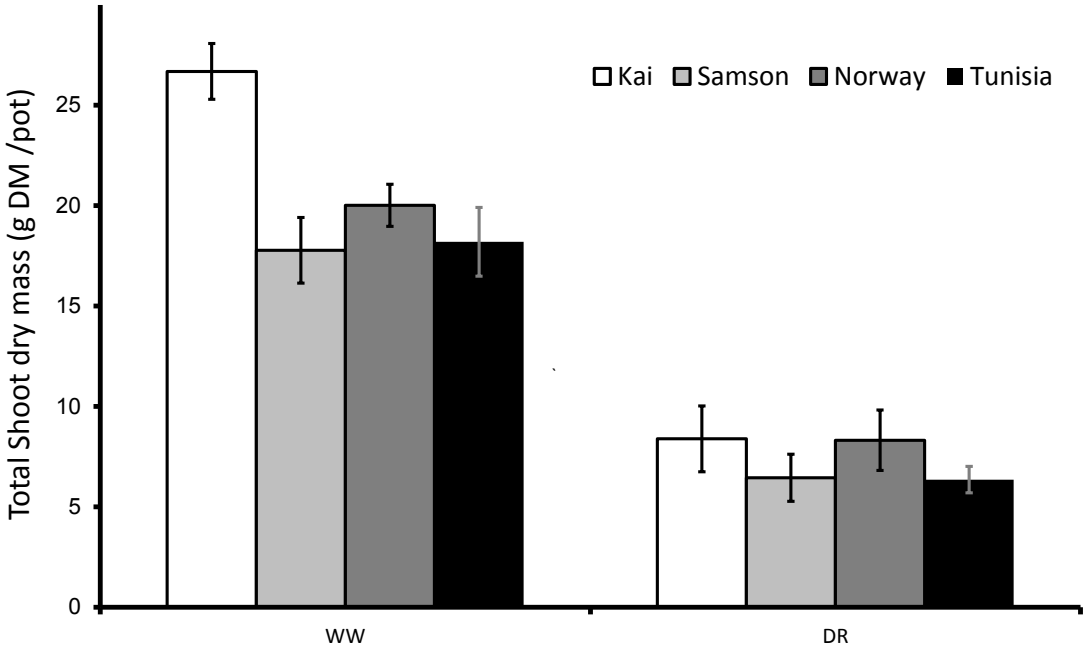

Figure 4-2. Shoot biomass of four perennial ryegrass populations following the second 28-day drought cycle. Water treatments: Well-watered (WW) and drought (DR). Error bars are ± SE.

4.1.3 Leaf dry matter production

Following the first drought cycle, in the well-watered pots, the Kai population had 67% larger leaf DM accumulation than all of the other populations ($P < 0.05$; Figure 4-3). In the water-stressed pots, all populations exhibited leaf DM reductions, but there were no differences among populations in terms of final leaf dry mass (Table 4-1). There were similar patterns after the two drought cycles for leaf DM reduction of the four populations with a non-significant harvest x treatment x population interaction. After both drought cycles, the leaf DM for Kai was reduced in the drought treatment to a greater extent (-70%) than in any other population.

After the second drought cycle, the general pattern remained unchanged for well-watered pots with the Kai population producing 31% more leaf dry mass than the others, while Samson had 13% greater leaf DM production than Tunisia ($P < 0.05$; Figure 4-3). The leaf DM of the Tunisian population was reduced to a lesser extent (-58%) by the water deficit than in the Kai population (-70%) after the second drought cycle (Table 4-1).

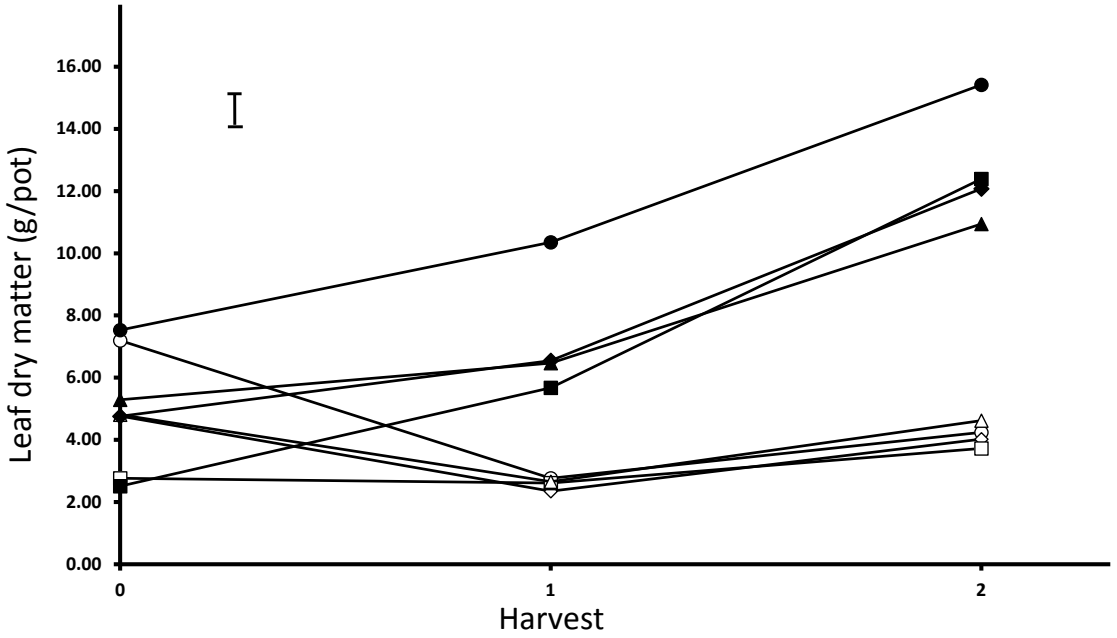

Figure 4-3 Leaf dry matter of four perennial ryegrass populations prior to the first drought cycle, following the first drought cycle and following the second drought cycle. *Lolium perenne* L. populations: Kai: WW (●), DR (○); Samson WW (■), DR (□); Norwegian WW (◆), DR (◇); Tunisian WW (▲), DR (△).The bar is the LSD $_{P<0.05}$ for the harvest x treatment x population interaction.

4.1.4 Leaf extension rate

Averaged across populations, leaf extension rate was more reduced by the first drought cycle (-75%), compared to the second drought cycle (-49%) ($P < 0.01$) (Figure 4-4 and Figure 4-5). Leaf extension in the four population was similarly affected by drought when comparing the two drought cycles (non-significant harvest x treatment x population interaction). After the first drought cycle, well-watered Kai plants had a greater mean LER (leaf extension rate) than those of the Norwegian population by 24% and the Tunisian population by 35%. The mean LER for Samson was 16% greater than that of the Tunisian population (Figure 4-4). The drought treatments reduced the mean LER of all populations after the first drought cycle (Table 4-1). Samson retained a higher mean LER than Kai by 111% and Norway 175% under the water deficit (Figure 4-4). Kai (-84%) and Norway (-85%), exhibited greater drought-generated reductions in LER than Samson (-62%) and Tunisia (-65%) during the first treatment period (Table 4-1 and Figure 4-4).

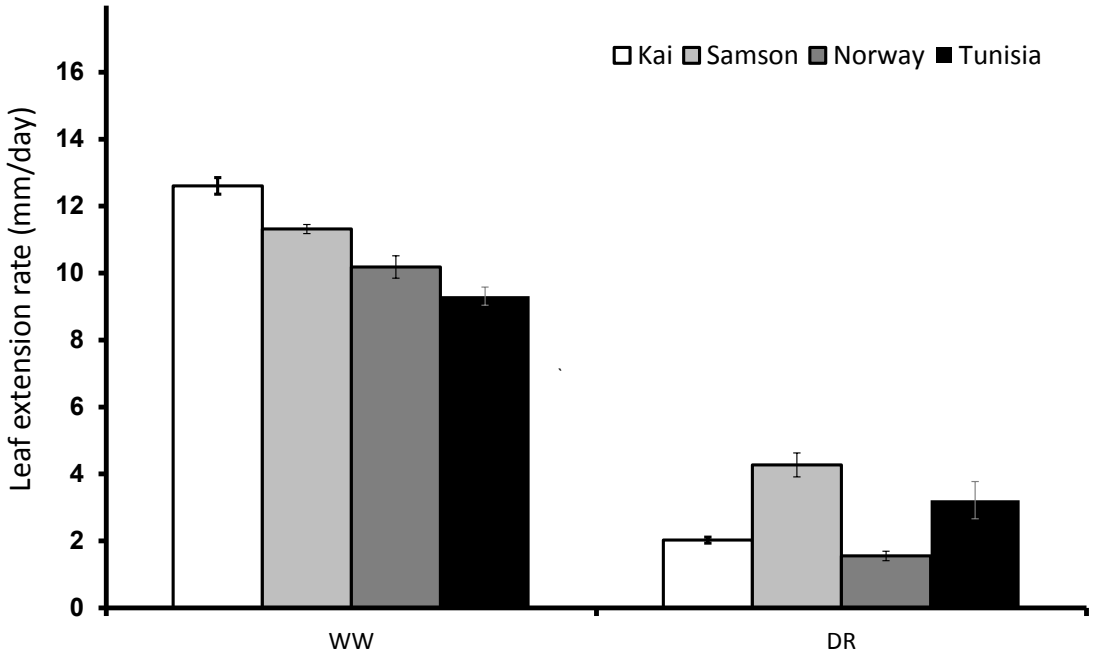

Figure 4-4 Mean leaf extension rate of four perennial ryegrass populations during the first 28 day drought cycle. Water treatments: Well-watered (WW) and drought (DR). Error bars are ± SE.

In the second drought cycle, well-watered Kai and Samson populations had greater mean LERs than that of the ecotypes by 43% and 29% respectively (Table 4-1 and Figure 4-5). In the water-stressed pots, all populations had reduced mean LER's (Table 4-1). Under the water deficit Samson had a higher mean LER than Norway and Kai by 28% and 26% respectively and the Tunisian population had a greater mean LER than the Norwegian and Kai populations by 50% and 47%, respectively. The mean LER of the Tunisian population was less affected by the drought (-23%), than all other populations. Kai was the most affected by the water deficit, exhibiting a reduction in LER of 64% (Table 4-1 and Figure 4-5).

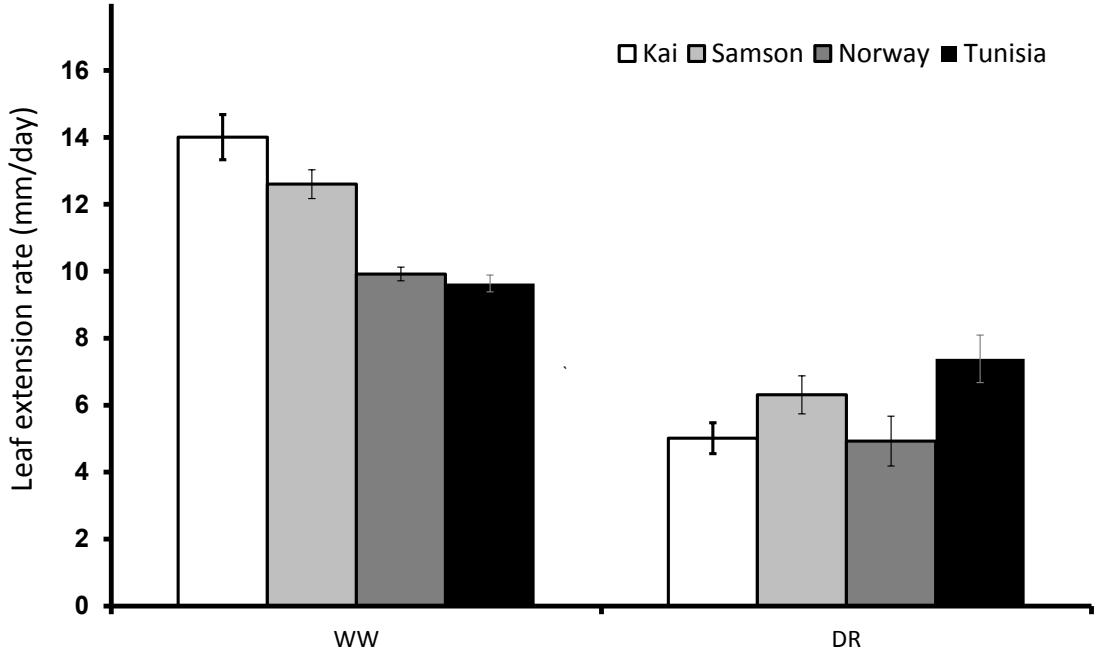

Figure 4-5 Mean leaf extension rate of four perennial ryegrass populations during the second 28 day drought cycle. Water treatments: Well-watered (WW) and drought (DR). Error bars are ± SE.

4.1.5 DM Stubble dry matter

In the well-watered pots, Kai had a stubble weight that was 56% larger than the mean of all other populations (Table 4-2). Additionally in the well-watered treatment, the Norwegian and Tunisian populations had higher stubble DM than that of Samson by 35% and 34% respectively. In the drought treatment, all populations exhibited a significant decrease in stubble DM (Table 4-1). All populations had comparable stubble DMs under the water deficit. However Kai was reduced to a greater extent by the drought (-65%) than Samson and Norway (-50% for both).

Table 4-2. Stubble dry matter of four perennial ryegrass populations following the two 28 day drought cycles.

Population	Well-watered	Drought
Kai	10.98 ± 0.44	3.82 ± 0.32
Samson	5.71 ± 0.49	2.87 ± 0.19
Norway	7.69 ± 0.48	3.87 ± 0.37
Tunisia	7.65 ± 0.62	3.27 ± 0.14

4.1.6 Root biomass production

Following the two drought cycles, in well-watered pots, Kai had a larger root DM than the Samson population by 62% and the Norwegian population by 44% ($P < 0.05$) (Table 4-3). In the drought treatment, the root DM of all populations was reduced similarly (Table 4-1).

Table 4-3. Root dry matter of perennial ryegrass populations following the two 28 day drought cycles.

Population	Well-watered	Drought
Kai	7.35 ± 1.18	3.05 ± 0.67
Samson	4.54 ± 0.68	2.36 ± 0.3
Norway	5.09 ± 0.65	2.05 ± 0.22
Tunisia	5.89 ± 0.72	3.66 ± 0.4

4.1.7 Root: shoot ratio

There was no difference in RSR (root to shoot DM ratios) between populations in the well-watered treatments, which averaged 0.27 (Figure 4-6; Table 4-1). Tunisia, Samson and Kai increased their RSR by 88%, 65% and 53% respectively in response to drought, while there was no change in the RSR of the Norwegian population (Figure 4-6; Table 4-1). Following these increases, analysis of the $LSD_{P<0.05}$ showed that the Tunisian population had a greater RSR than the Norwegian population by 97% and Samson by 41% in the drought treatment.

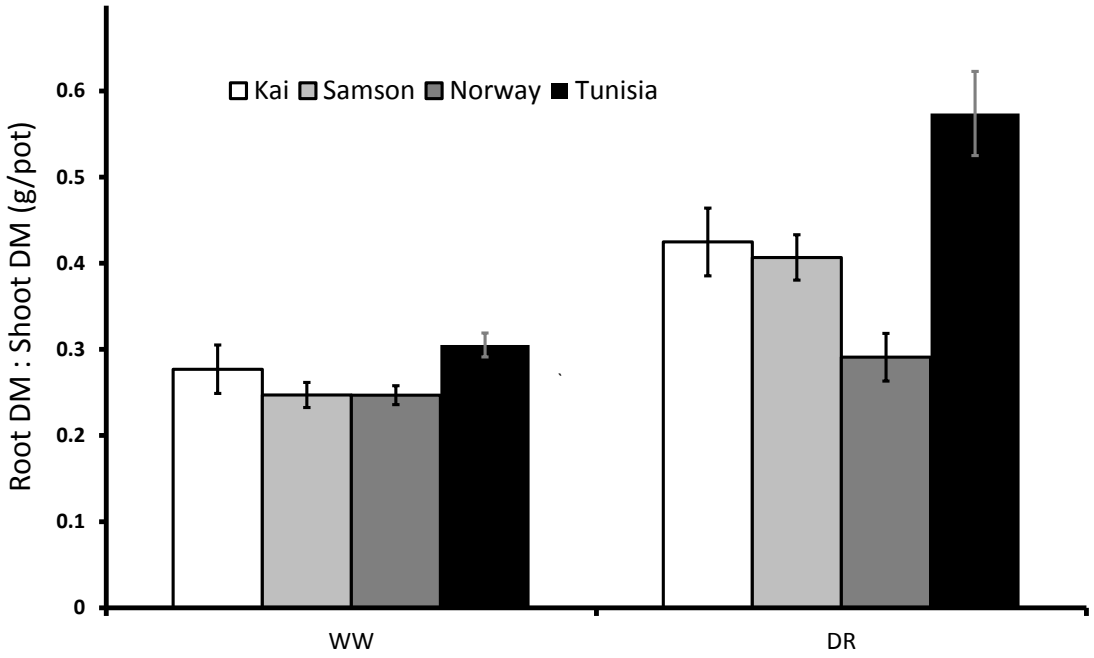

Figure 4-6. Root to shoot dry matter ratio for perennial ryegrass populations following the second 28 day drought cycle. Water treatments: Well-watered (WW) and drought (DR). Error bars are ± SE.

4.1.8 Relative water content

After the second drought cycle, drought had halved leaf RWC across populations to an average of 47% from 92% in the well-watered pots. (Figure 4-7). However, in the drought treatment, the RWC for the Norwegian population (60%) was greater than that of Kai (35%, $LSD_{P<0.05}$) (Table 4-1).

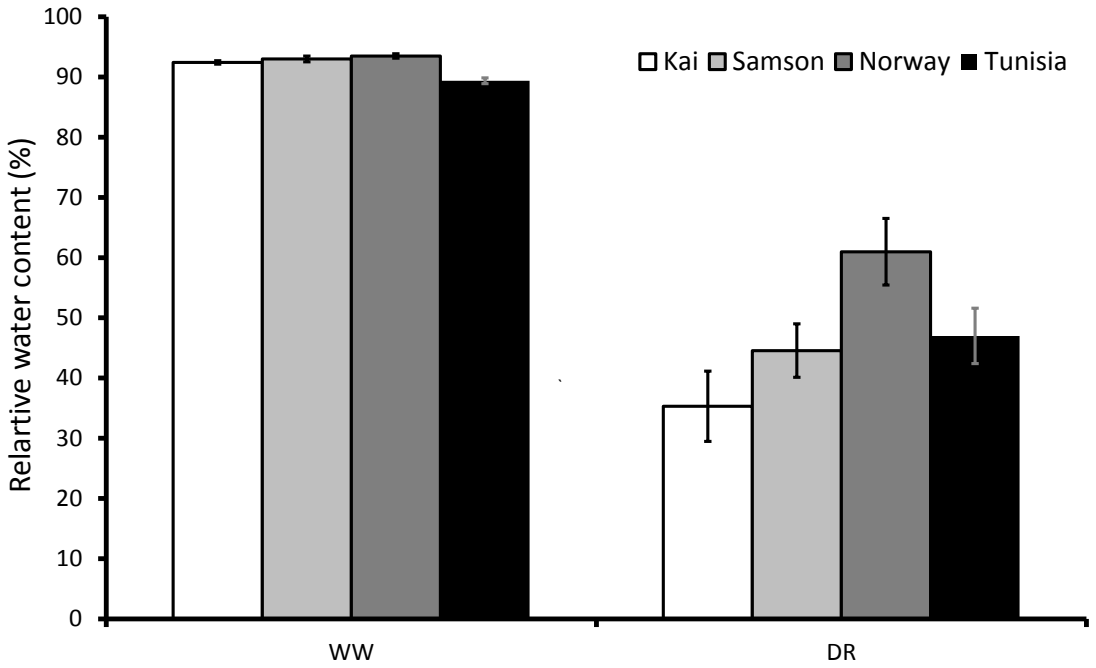

Figure 4-7 Relative water content of perennial ryegrass populations following the second 28 day drought cycle. Water treatments: Well-watered (WW) and drought (DR). Error bars are ± SE.

4.1.9 Solute potential

The adjusted Ψ_s (solute potential) did not differ between any of the populations in the well-watered pots (Figure 4-8; Table 4-1). However the drought treatment caused a reduction in Ψ_s in the Kai, Samson and Tunisian populations by 145%, 139% and 92% respectively. In contrast to this the Norwegian population exhibited no change in Ψ_s between well-watered replicates and water-stressed replicates. In the drought treatment, the Norwegian population had a more positive Ψ_s (-1.66 MPa) in comparison with the other populations. Kai had the most negative Ψ_s (-4.09 MPa) in the drought treatment in comparison with the other populations.

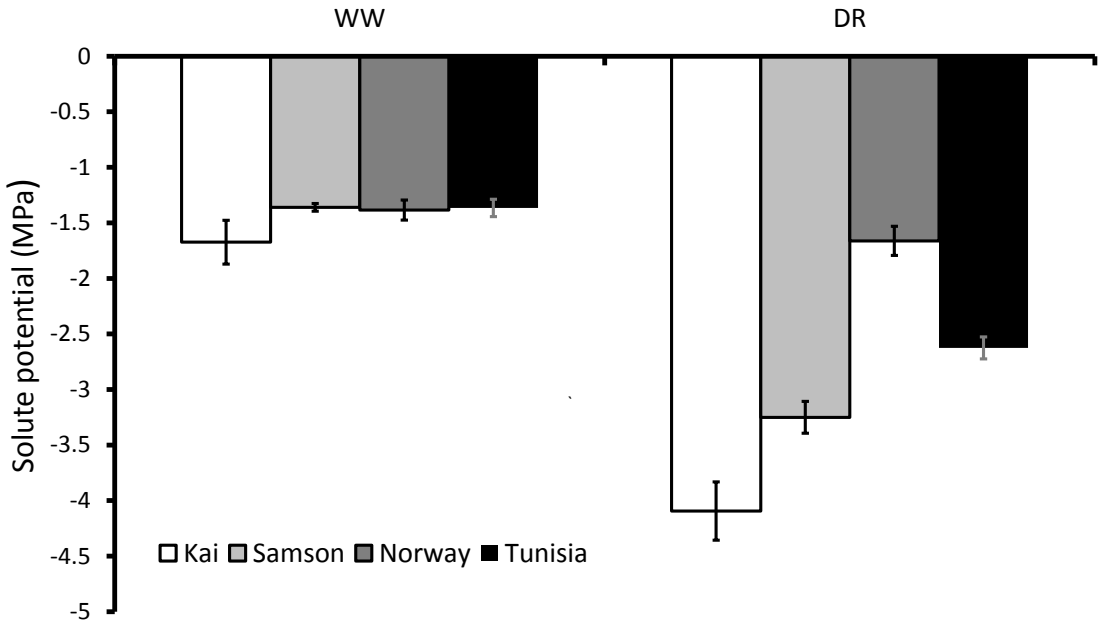

Figure 4-8 Solute potential of perennial ryegrass populations following the second 28 day drought cycle. Water treatments: Well-watered (WW) and drought (DR). Error bars are ± SE.

4.1.10 Attributes related to photosynthesis

The canopy temperature did not differ between any populations under either water treatment, however all populations showed a similar increase in canopy temperature under drought (Table 4-4; Table 4-1).

In the well-watered pots the Tunisian population had a greater SPAD value than that of Kai by 23% ($LSD_{P<0.05}$; Table 4-4). The drought treatment caused an increase in the SPAD values of all populations (Table 4-1), but there were no differences in SPAD values between populations in the drought treatment.

Table 4-4 Canopy temperature and SPAD values for perennial ryegrass populations. Water treatments: Well-watered (WW) and drought (DR).

Population	Canopy Temp WW	Canopy Temp DR	SPAD WW	SPAD DR
Kai	22.28 ± 0.56	28.55 ± 0.5	34.72 ± 1.7	42.37 ± 3.39
Samson	22.65 ± 0.65	29.65 ± 1.01	38.92 ± 1.66	43.92 ± 1.91
Norway	22.58 ± 0.44	27.95 ± 1.33	38.53 ± 1.13	45.80 ± 1.84
Tunisia	22.45 ± 1.04	30.27 ± 1.17	42.60 ± 3.08	45.93 ± 2.3

4.1.11 Endophyte presence

The endophyte screening process revealed that the Kai, Samson and Norwegian populations were endophyte negative with zero positive stains out of 15 replicate seedling blots. The Tunisian population was endophyte positive with 11 positive stains from 15 replicate seedling blots.

4.2 Correlations

There was a negative relationship between Ψ_s of perennial ryegrass leaves under drought and the extent of reduction in shoot biomass (Figure 4-9). The large reduction in Ψ_s of Kai in the drought treatment was accompanied by a large reduction in total shoot biomass, whereas the Norwegian population with a smaller reduction in Ψ_s in the drought treatment had a relatively lesser reduction in total shoot biomass. Intermediate responses in reductions in both Ψ_s and shoot biomass were exhibited by the Samson and Tunisian populations. This relationship was more significant ($P<0.05$) when the regression was calculated from the individual data values for Ψ_s and total shoot biomass (Figure 6-1).

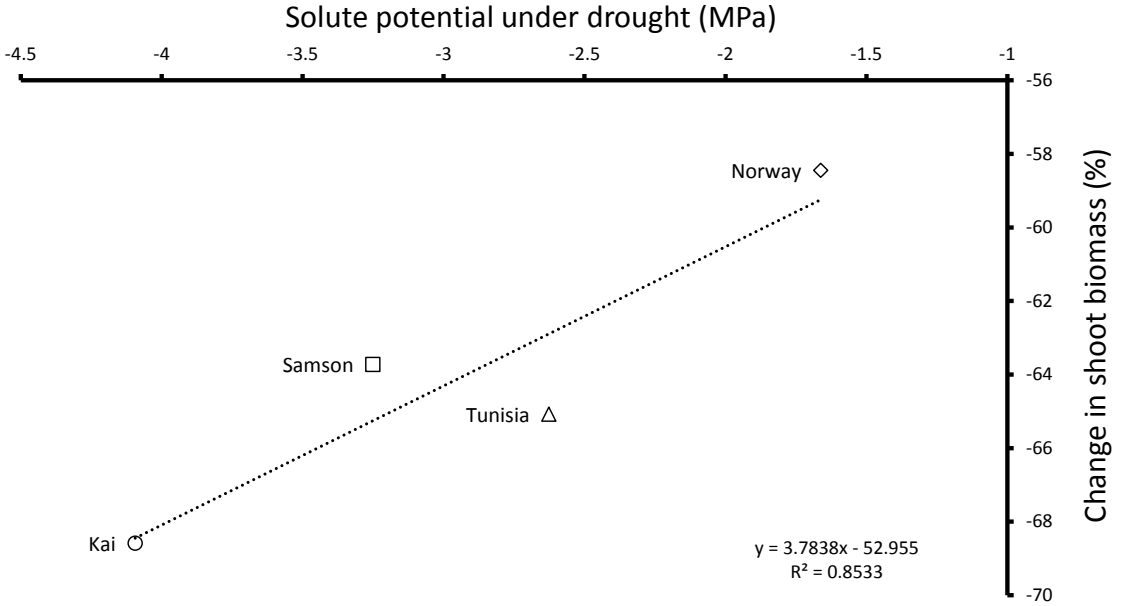

Figure 4-9 Regression of the drought-induced change in total shoot biomass of four perennial ryegrass populations under drought against their solute potential under drought.

A positive relationship was discovered between the change in canopy temperature and the change in the root to shoot DM ratio under drought (Figure 4-10), highlighting that an increase in leaf temperature was reflected in an increased root: shoot ratio.

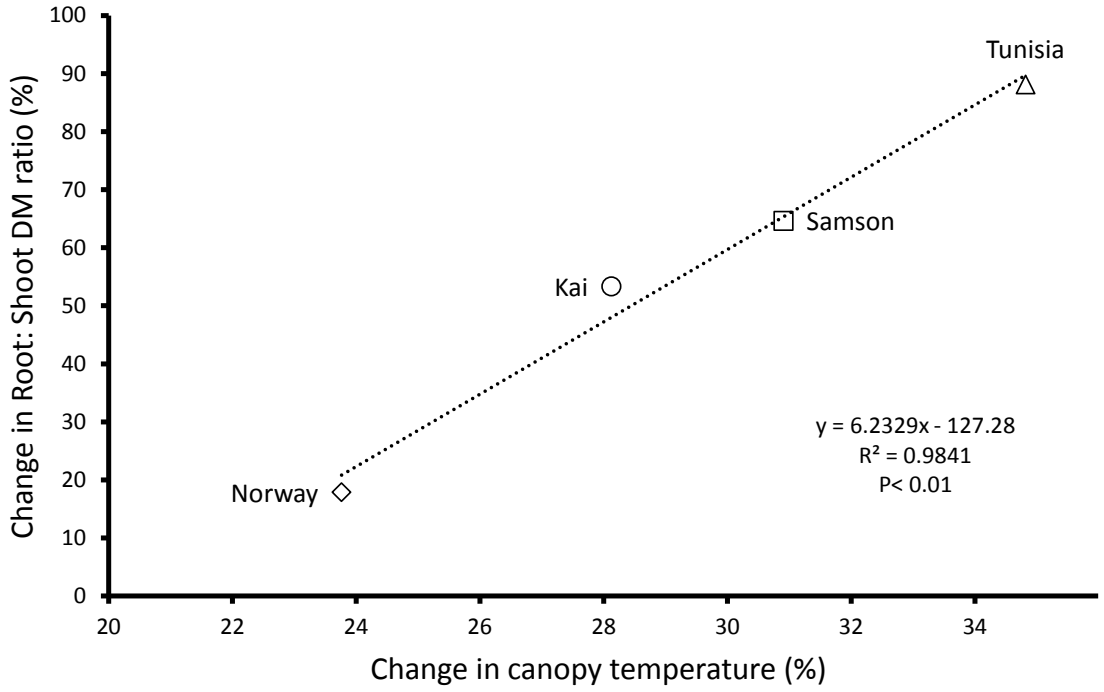

Figure 4-10 Regression of drought-induced change in the root to shoot dry weight ratio of four perennial ryegrass populations against the drought induced change in their canopy temperature.

There was a positive relationship between the root DM and the root to shoot DM ratio under drought (Figure 4-11), emphasizing that higher maintenance root DM under drought was reflected in an increase in the root to shoot DM ratio. This relationship was more significant ($P<0.05$) when a regression was produced from the individual data values for root DM and root to shoot DM ratio (Figure 6-2).

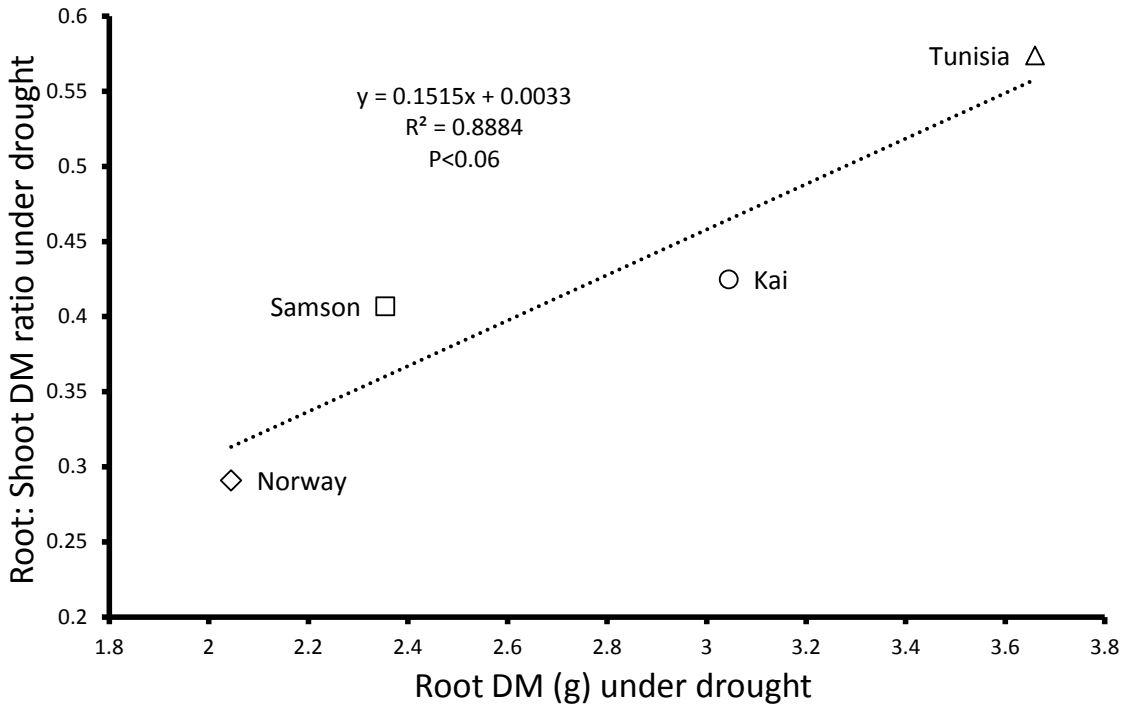

Figure 4-11 Regression of the root to shoot DM ratio of four perennial ryegrass populations under drought against their root dry weight under drought.

A negative relationship was discovered between the RWC and the change in total shoot biomass in response to drought (Figure 4-12). That is, the higher the RWC under drought, the lesser the reduction in shoot biomass under drought: the higher RWC of the Norwegian population under drought was reflected in reduced drought-generated loss of shoot biomass in that population in comparison with Kai, which had a low RWC under drought (Figure 4-12).

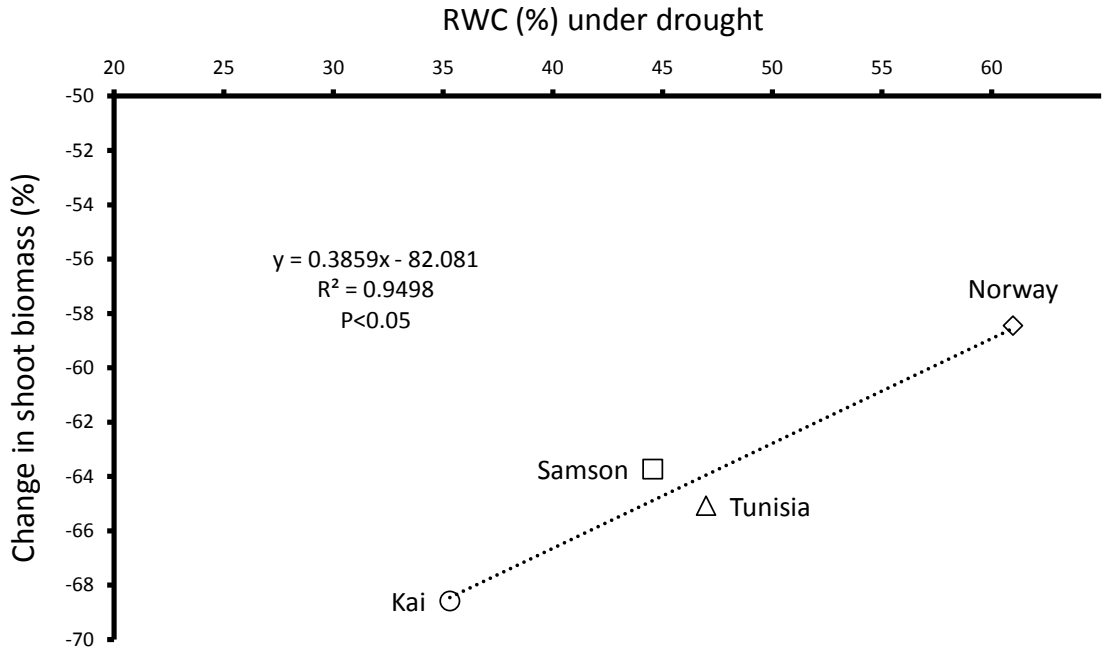

Figure 4-12 Regression of the drought-induced change in total shoot biomass of four perennial ryegrass populations against their relative water content under drought.

A negative relationship was also found between estimated chlorophyll concentration (SPAD) under well-watered conditions and the drought-induced change in the mean leaf extension rate in the second drought cycle (Figure 4-13). This highlighted that higher leaf chlorophyll concentrations prior to drought resulted in lesser reductions in mean leaf extension rates under drought.

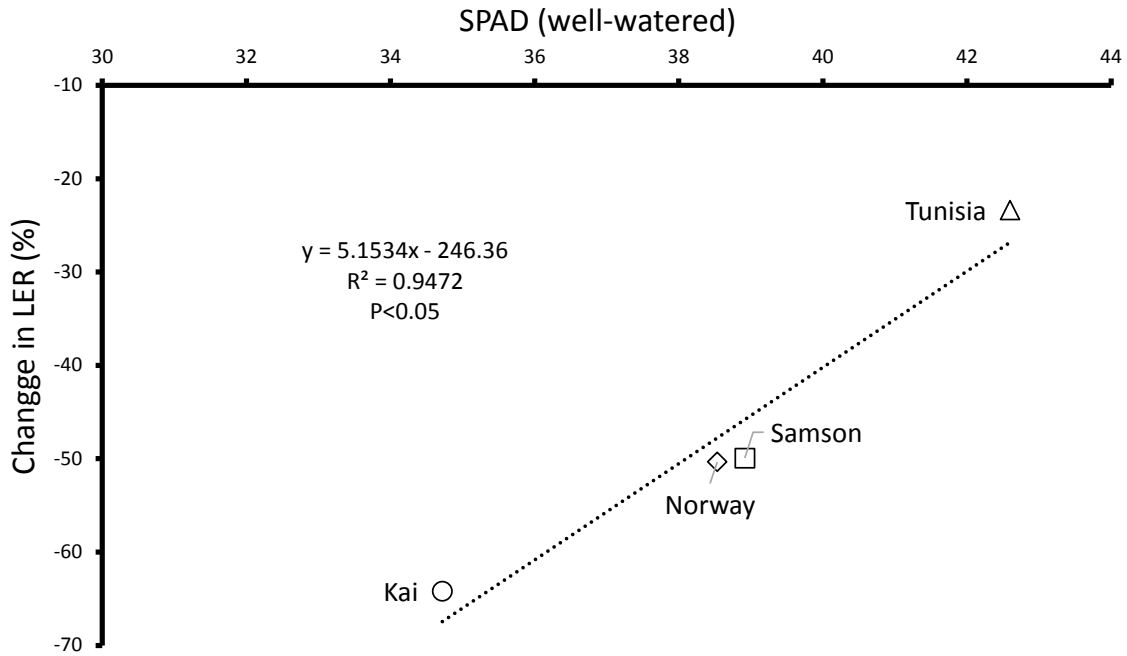

Figure 4-13 Regression of the drought induced change in leaf extension rates of four perennial ryegrass populations against their estimated chlorophyll concentration under well-watered conditions.

5 Discussion

5.1 Above-ground dry matter yield

This study investigated the early morphological and physiological responses of four perennial ryegrass populations under a controlled drought. For a grazed-pasture system, total shoot biomass or above ground dry matter yield is a common measure of plant performance (Easton et al. 2001) and in this work it was the sum of leaf and stubble dry mass yield. The bred populations were noticeably more productive than the ecotypes under well-watered conditions, as also found by Hussain (2013), where Samson yielded greater than a Mediterranean ecotype. Kai performed particularly well in favourable conditions with a total shoot biomass that was superior to that of the other populations (Figure 4-2). This could be attributed to higher LERs over the two drought cycles, heavier stubble and a larger leaf biomass at the commencement of the first drought cycle. Despite maintaining comparable LERs to the Kai population and making rapid gains in leaf dry matter production throughout the trial, the productivity of the Samson population was initially delayed by its later establishment. This resulted in the Samson plants producing similar shoot biomass and green leaf yields to the ecotypes during and at the conclusion of the two-month experimental period.

The ecotypes; from Norway and Tunisia consistently under-performed in key production traits in comparison with the bred populations under favourable conditions. An example of this was the difference in the mean LERs over both droughts, where the bred cultivars were clearly superior to the ecotypes (Figure 4-4; Figure 4-5). The inability of the ecotypes to produce large yields under favourable conditions would certainly limit their ability to serve a functional role in a grazed New Zealand pasture.

In the drought treatment the total shoot biomass was reduced to comparable levels amongst the four populations (Figure 4-2). This indicates that any advantages that the bred populations have in terms of the ability to produce high dry matter yields, were negated by the lack of water. Reductions in shoot biomass under drought were also corroborated by reductions in LERs, leaf dry matter production and stubble dry mass across the populations. The mean shoot dry matter reduction across the four populations was similar to the reduction in perennial ryegrass herbage yield under drought stress found by (Korte & Chu 1983). Relative to the highly productive well-watered plants, the shoot biomass of the Kai population was reduced to the greatest extent by drought. In contrast, shoot biomass of the Norwegian population was reduced the least by drought. This was a reflection of its lower shoot biomass under well-watered conditions, as well

as relative maintenance of stubble dry matter under drought in comparison with Kai (Table 4-2). The latter indicates advantages for the Norwegian population for stubble regrowth after drought.

The loss of shoot biomass under drought in the Tunisian population was reflected in a concomitant loss in stubble weight (Table 4-2). However the Tunisian population was able to maintain the same leaf dry matter production under drought as other populations in the first drought cycle and surpass the leaf dry matter production of Norway and Samson in the second drought cycle (Figure 4-3). This could be attributed to the strong mean LER of the Tunisian population in the first drought cycle and a relatively higher mean LER over the second drought cycle (Figure 4-4; Figure 4-5).

While slow establishment impeded the ability of Samson to grow a comparable biomass to Kai under well-watered conditions, this likely improved its fitness for survival, particularly in the first drought phase. By obtaining a smaller initial leaf area (characterised by the low initial leaf dry mass) than the other populations, water loss from the pot via transpiration would have been limited. Additionally the smaller size of the Samson plant in general, likely meant that at the commencement of the first drought, Samson plants had used relatively less of the water in their pots in comparison with the other populations. This was reflected in the Samson population obtaining the highest mean LER over the first drought cycle out of all of the populations (Figure 4-4).

5.2 Root biomass

The aptitude of a root system to supply a plant with water is related to its mass and is an important trait for pasture plants commonly exposed to drought (Comas et al. 2013). The comparison of shoot (Figure 4-2) and root biomass (Table 4-3) under well-watered conditions suggested that a similar proportion of photoassimilates was being transported from the shoots to roots across the populations. Under drought however, our data suggest that a higher root to shoot DM was a function of maintaining higher root DM levels (Figure 4-11). This is in line with the capacity of perennial ryegrass to shift assimilate supply to different plant parts depending on sink requirements (Thomas & Davies 1977). The drought-induced increase in the root to shoot DM ratio of the Tunisian population was greater than both of the Kai and Samson populations (Figure 4-6). This indicates that the Tunisian population began to implement a plant strategy to focus resources under drought towards maintenance of root growth as opposed to aboveground productivity (see further discussion of the ecotype responses in section 5.4 below).

5.3 Physiological traits

The relative water content of leaves is a useful indicator for the level of stress a drought-exposed plant is experiencing (Ganji Arjenaki et al. 2012) and an important trait determining drought tolerance in cool-season grasses (Abraham et al. 2004). Comparable and high RWC levels among the four populations in the well-watered treatment as well as a reduction in RWC under drought was expected (Figure 4-7). The reduction under drought from a mean RWC of 92% down to 47% across the populations was much larger than found elsewhere (Jiang et al. 2009) where the RWC of perennial ryegrass leaves was reduced from 94.5% to 71.5%. However soil moisture contents were much higher in the drought pots of that study, compared to this work.

The capacity to maintain a higher RWC under water stress was related to the extent at which total shoot biomass was reduced in the perennial ryegrass populations (Figure 4-12). The Norwegian ecotype had higher RWC than Kai under drought and subsequently exhibited a smaller loss of total shoot biomass in the drought treatment. A higher RWC enables the plant to maintain turgidity in vital tissues and therefore sustain function and preserve biomass under stress (Blum 2002).

The reduction in Ψ_s via osmotic adjustment was expected as it is an important protective response of perennial ryegrass under drought (Thomas 1990). All populations excluding Norway carried out such osmotic adjustment, with Kai reducing its Ψ_s the most (Figure 4-7). This was a comparatively larger shift than was found by Thomas (1991) in perennial ryegrass plants, with Ψ_s of -2.6 and -2.8 MPa after eight weeks of drought. Tetraploid perennial ryegrass has been found to have a lower osmotic potential than diploid perennial ryegrass (Sugiyama 2006). The lack of osmoregulation in the Norwegian population indicated that it was not in a situation where it needed to counteract the drought-induced reduction in cell water potential. This suggests that the Norwegian ecotype was saving water elsewhere and this will be discussed below.

The lack of variance in canopy temperature among the four populations was anticipated for the well-watered treatment, but not the drought treatment. Canopy was used here as a proxy for stomatal conductance, a key early water-saving response mechanism under drought (Tombesi et al. 2015). The average canopy temperature for the populations in the drought treatment was 29.1 °C, lower than the canopy temperature of ryegrass under arid conditions of 32.4 to 35.7°C found by (Franca et al. 1998). However in that work, mean daily air temperatures were consistently around 25°C which would have exacerbated canopy temperature rises. Under drought, the increase in canopy temperature was an indication that plants were attempting to limit water loss through increased stomatal resistance, reducing transpiration (Jackson et al. 1981).

The difference in chlorophyll concentration between the well-watered Tunisian population and Kai could be a result of larger cells in tetraploids, effectively 'diluting' chlorophyll concentration (Sugiyama 2006). Decreases in the chlorophyll content of perennial ryegrass under drought have been reported (Alberte et al. 1977; Jiang et al. 2009). However in the current study, chlorophyll concentration increased in water-stressed plants. This is likely the direct result of cell dehydration leading to an increase in the relative amount of chlorophyll per unit area of the leaf. This also demonstrates that the drought plants were not stressed to a point where chlorophyll degradation by oxidation was beginning to occur.

The correlation between SPAD levels and mean leaf extension rate across the populations indicated that constitutively high chlorophyll or nitrogen concentration in leaves has a significant bearing on leaf expansion processes under a water deficit (Figure 4-13). Leaf expansion is particularly sensitive to nutrient supply due primarily to the requirement for synthesis of new materials (Atwell et al. 1999). Nitrogen supply has been reported to have a significant effect on leaf expansion by affecting both cell expansion and cell division by influencing chlorophyll and rubisco concentration (Murchie & Horton 1997; Justes et al. 2002). The advantage of this for LER is plausible in that a higher leaf chlorophyll concentration is likely to have allowed plants to carry out a higher rate of photosynthesis for leaf growth for the same amount of water as a plant with lower chlorophyll concentration (Shangguan et al. 2000).

5.4 Drought tolerance strategies of ecotypes

Both perennial ryegrass ecotypes showed early indications of tolerance against water deficit, whilst differing in their drought tolerance strategies. The water-saving strategy of the Norwegian population was based mainly around early morphological adaption, where leaf extension was halted. This would have strongly reduced transpiration rates under drought, thus saving water and enabling photoassimilates to be used and transported for growth and maintenance (van der Wal et al. 1975). It appears that the other populations instead consumed the resources to maintain root dry mass and more importantly to manufacture osmolytes for the preservation of growth processes, whereas the Norwegian ecotype was able to save the bulk of the energy for survival.

The negative relationship between Ψ_s and reduction in shoot biomass under drought (Figure 4-9) confirms that production of osmolytes is expensive to the plant in terms of carbon consumption (Zamski & Schaffer 1996). By spending resources on osmoregulation, the Kai population, in particular, is likely to have diverted a large amount of resources away from growth, which is likely to have contributed to its pronounced loss of shoot biomass under drought. Selection for osmotic

adjustment can thus often reduce dry matter yield under drought (Grumet et al. 1987). By not spending resources on producing osmolytes, it can be suggested that the Norwegian population can store them or utilise them elsewhere.

By conserving resources the Norwegian ecotype thus went into a preservation stage where leaves were simply 'kept alive' and drought-induced reduction of stubble mass was less pronounced. This was achieved by maintaining high relatively high RWC (Figure 4-7) under drought to limit the damage caused to cellular components that often occurs in wilted leaves (Fu & Huang 2001). Perennial ryegrass is more susceptible to membrane damage in warm, dry conditions compared to tall fescue (Jiang & Huang 2001). Relative maintenance of stubble material (Table 4-2) under drought in the Norwegian population reflects higher preservation of tillers. By ensuring their survival through the drought phase, enhanced regrowth upon re-watering, is possible from vital apical meristems. Increased tiller survival was one reason given for increased pasture production following relief from drought (Brougham 1970). This is a key driver for increased persistence in perennial ryegrass over long, dry summers (Korte & Chu 1983) and provides a possible explanation for preliminary findings of long-term persistence of the Norwegian germplasm (Cyriac 2016).

The root to shoot DW ratio was increased by drought in all populations apart from Norway, which demonstrated that it was not affected by the drought stress to an extent where increasing root growth relative to the shoot was a priority such as in the population from Tunisia (Figure 4-6). The latter ecotype exhibited a drought response strategy that contrasted with that of the Norwegian population in that it made physiological and morphological changes that allowed for the increased acquisition of water to maintain shoot growth.

An increased root to shoot DM ratio under drought is often accomplished via a sharp decline in shoot biomass, as opposed to root growth (Sharp et al. 2004). However, leaf extension of the Tunisian population was reduced to the lowest extent of the four populations in the second drought period. Furthermore among the perennial ryegrass populations, the Tunisian ecotype had the largest root to shoot DM ratio under drought and this was related to a relative maintenance of root dry mass under drought (Figure 4-11), as opposed to a loss of shoot biomass. This is likely the result of increased partitioning of assimilates for the growth of below-ground structures to maintain water uptake (Irving 2015) and represents a key drought tolerance mechanism (Levitt 1980). The relationship between drought-induced changes in canopy temperature and changes in the root to shoot DW ratio (Figure 4-10) indicated that water conservation from increased stomatal resistance under drought allowed the redirection of resources towards relative maintenance of root – rather than shoot – growth. Taken together,

these findings suggest morphological and physiological advantages for the Tunisian ecotype with regard to the conservation and efficient utilisation of water.

There is also a likely connection between the presence of endophyte in the Tunisian population, its improved leaf growth and increased root to shoot DW ratio under the drought treatment in comparison with the other populations, which were endophyte negative. Endophyte-positive perennial ryegrass accessions from Turkey had greater shoot mass than did endophyte-negative plants of the same accession under drought (Kane 2011). In other work (Hesse et al. 2003), endophyte infected perennial ryegrass accessions had increased root DWs and root to shoot DW ratios. It has been suggested that endophyte infection affects source-sink relationships in the plant that favours root growth (Hesse et al. 2003). In endophyte-infected perennial ryegrass plants, drought tolerance genes which included root growth, may be upregulated under drought (Zhou 2013). However the relationship between endophytes and drought tolerance in perennial ryegrass remains poorly understood (Zhou 2013).

5.5 Implications and recommendations for future research.

While this work has uncovered some key response mechanisms to drought, there were some areas that could be improved should the experiment be repeated. A higher number of drought cycles would be useful to examine drought responses of perennial ryegrass after longer periods of desiccation stress (Cyriac 2016). All perennial ryegrass populations were not of the same maturity and biomass before the first drought cycle which led to Samson being affected differently by the drought in comparison with the other populations.

A future experiment should be conducted in the field. During summer, temperatures would likely exceed 30°C, leading to increases in transpiration and thus affecting the water relations of the perennial ryegrass populations differently than the drought treatment in this experiment. Under field conditions, plant roots would not be confined to a pot and may be able to extend deeper into the soil profile to access water. In this instance, the measurement of more root-related traits would be practical. Additionally, grazing and treading of plots will indicate the practicality of using germplasm based on these ecotypes for grazed systems. In many instances the latter would also include legumes which would test the competitive capacity of the ecotypes in a sward. The effect of endophyte as another treatment factor would provide further value in such studies.

5.6 Conclusions

The main findings of the study were:

(i) There were intraspecific differences in key morphological and physiological drought responses in perennial ryegrass.

Both the Norwegian population (reduced leaf area and conservation of resources) and Tunisian population (increased root: shoot DM ratio) displayed drought response strategies of value for long-term persistence, after only two drought cycles. In contrast, the bred material only produced short-term responses to drought. Therefore the ecotypes appear to possess an improved ability for preparing themselves for longer drought periods, which would increase their chances of maintaining production or persistence in a pasture under long term-drought conditions.

(ii) Drought response strategies varied between ecotypes and may boost dry matter yield under long-term drought.

The two contrasting drought response strategies in the ecotypes could both have positive implications for perennial ryegrass productivity in drylands. The maintenance of resources which other populations direct towards osmotic adjustment could mean that in the Norwegian population, primary metabolites such as sugars and amino acids will be available for growth and dry matter production under extended periods of drought. The relative maintenance of root growth under drought by the Tunisian population can translate into maintenance of leaf growth in extended drought phases. Furthermore, the presence of endophyte in the Tunisian population probably played a role in the increased partitioning of resources for the maintenance of root growth under drought.

Taken together, the findings provide functional information that can be used in future breeding initiatives towards improving drought tolerance in perennial ryegrass.

Acknowledgements

I would like to dedicate this dissertation to my late grandfather Donald Arthur Wilson.

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6 Appendix

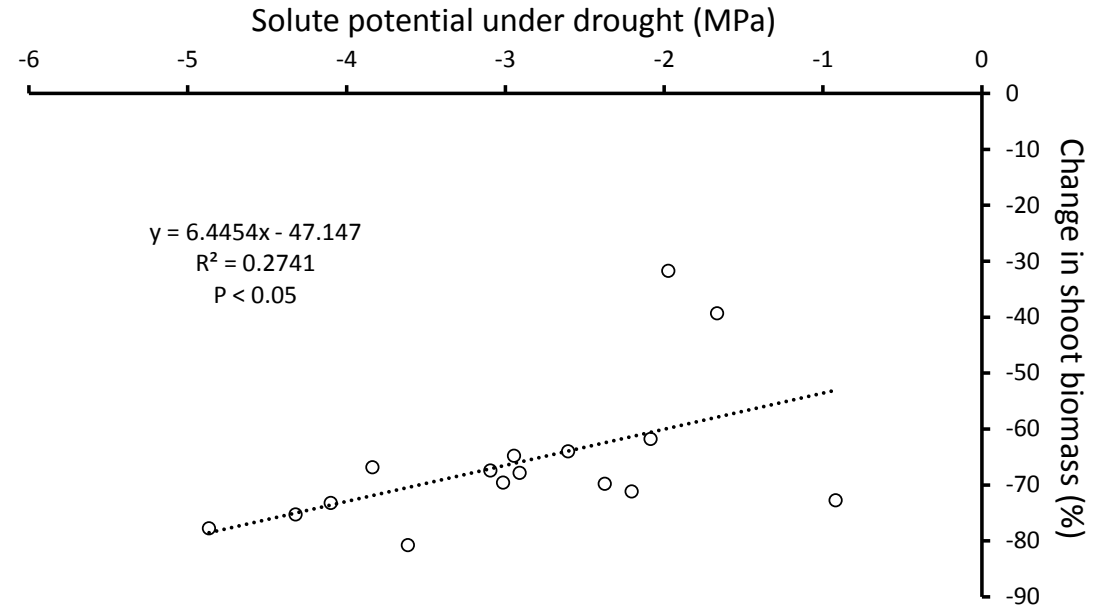

Figure 6-1 Regression of the individual data points for the drought-induced change in total shoot biomass of four perennial ryegrass populations against their solute potential under drought.

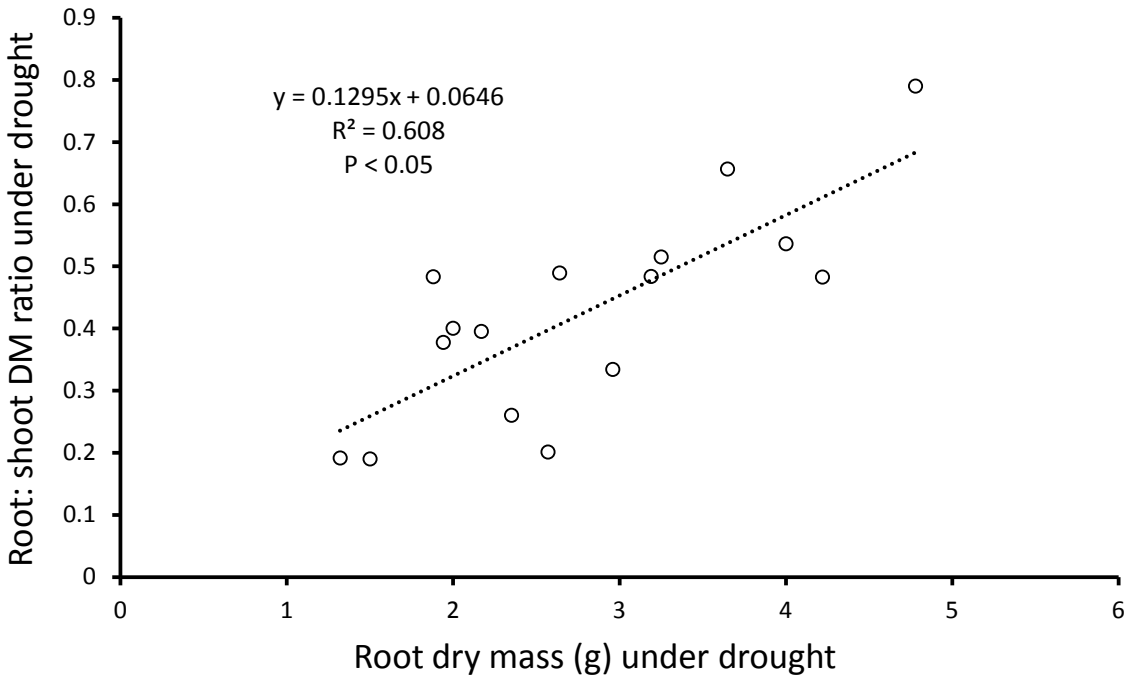

Figure 6-2 Regression of the individual data points for the root to shoot dry matter ratio of four perennial ryegrass populations under drought against their root dry matter under drought.